Diversification of sympatric broadcast-spawning limpets (*Cellana* spp.) within the Hawaiian archipelago

CHRISTOPHER E. BIRD,* BRENDEN S. HOLLAND,† BRIAN W. BOWEN* and ROBERT J. TOONEN*

*Hawai‘i Institute of Marine Biology, School of Ocean and Earth Sciences, University of Hawai‘i at Mānoa, PO Box 1346, Kāne‘ohe, HI 96744, USA, †Center for Conservation Research & Training, University of Hawai‘i at Mānoa, 3050 Maile Way, 408 Gilmore, Honolulu, HI 96822, USA

Abstract

Speciation remains a central enigma in biology, and nowhere is this more apparent than in shallow tropical seas where biodiversity rivals that of tropical rainforests. Obvious barriers to gene flow are few and most marine species have a highly dispersive larval stage, which should greatly decrease opportunities for speciation via geographic isolation. The disparity in the level of geographic isolation for terrestrial and marine species is exemplified in Hawai‘i where opportunities for allopatric speciation abound in the terrestrial realm. In contrast, marine colonizers of Hawai‘i are believed to produce only a single endemic species or population, due to the lack of isolating barriers. To test the assertion that marine species do not diversify within Hawai‘i, we examine the evolutionary origin of three endemic limpets (*Cellana exarata, C. sandwicensis* and *C. talcosa*) that are vertically segregated across a steep ecoclone on rocky shores. Analyses of three mtDNA loci (12S, 16S, COI; 1565 bp) and two nDNA loci (ATPSβ, H3; 709 bp) in 26 Indo-Pacific *Cellana* species (*N* = 414) indicates that Hawai‘i was colonized once ~3.4–7.2 Ma from the vicinity of Japan. Trait mapping demonstrates that high-shore residence is the ancestral character state, such that mid- and low-shore species are the product of subsequent diversification. The Hawaiian *Cellana* are the first broadcast-spawners demonstrated to have speciated within any archipelago. The habitat stratification, extensive sympatry, and evolutionary history of these limpets collectively indicate a strong ecological component to speciation and support the growing body of evidence for non-allopatric speciation in the ocean.

Keywords: colonization, ecological selection, evolution, speciation, trait mapping

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Introduction

The origins of marine biodiversity remain enigmatic, given the dispersive potential of most marine species. The world’s oceans present few impermeable barriers to the dispersal of aquatic species, most of which have a mobile life phase (Kinlan & Gaines 2003). Yet, the tropical Indo-Pacific Ocean harbors great biological diversity on shallow water reefs which rivals that of tropical rainforests. The explanations for this phenomenon generally include cryptic physical barriers and/or ecologically driven barriers to gene flow (Hellberg 1998; Dawson *et al.* 2002; Barber *et al.* 2006; Rocha & Bowen 2008). Allopatric speciation, where physical barriers block gene flow, is the most commonly documented mode of speciation in nature (Schluter 2009) and is considered by some to be the only plausible biogeographic mode of speciation (Mayr 1963). A growing body of evidence, however, supports sympatric speciation, where physical barriers do not prevent gene flow during speciation (Gavrilets *et al.* 2007; Nosil 2008b; Nosil *et al.* 2009; Rice & Pfennig 2010). Terrestrial examples of putative sym-
patric speciation include *Howea* palm trees (Savolainen et al. 2006; Babik et al. 2009) and *Gyrinophilus* cave salamanders (Niemiller et al. 2008), fresh water examples include the fish *Amphilophus* (Barluenga et al. 2006; Geiger et al. 2010) and *Gyrinophilus* cave salamanders (Niemiller et al. 2008), and marine examples include temperate *Hexagrammos* reef fish (Crow et al. 2010), tropical *Halichoeres* reef fishes (Rocha et al. 2005), *Seritopora hystrix* reef building coral (Bongaerts et al. 2010), intertidal *Littorina saxatilis* snails (Johannesson et al. 2010), *Fucus* macroalgae (Forslund & Kautsky 2009), and *Orcinus orca* killer whales (Foote et al. 2009). Because sympatric speciation is controversial, allopatric speciation is considered to be the default null hypothesis in investigations of speciation. Coyne & Orr (2004) state that four characteristics, at a minimum, must be demonstrated to conclude that sympatric speciation has occurred in a group of extant species: (i) sympatric distributions, (ii) a monophyletic evolutionary lineage, (iii) reproductive isolation, and (iv) an evolutionary and geographic setting where allopatric speciation is improbable.

The case of the Hawaiian limpets

Three endemic Hawaiian limpets, *Cellana exarata*, *C. sandwicensis* and *C. talcosa*, were singled out by Kay & Palumbi (1987) as a possible exception to the rule that marine species do not diversify within the Hawaiian Islands. The Hawaiian *Cellana* (Fig. 1), collectively known as ‘opihis, are broadcast-spawners with a 3–18 day pelagic phase before larvae are competent to settle on the shore and metamorphose (Corpu 1983). ‘Opihi are vertically stratified on wave-exposed shorelines, with *C. exarata* on the high shore, *C. sandwicensis* on the mid to low shore, and *C. talcosa* on the low shore to shallow subtidal zone (Kay & Magruder 1977; Bird 2006). Due to the modest tidal range in Hawai‘i, (0.6 m) all three species occur within centimeters of each other, sometimes overlap, and release their gametes into the high energy wave-wash (mean annual significant wave height ~1.9 m, 3 × the tidal range; Kay & Magruder 1977; Bird et al. 2007). *Cellana exarata* is known to spawn within the week following the new moon in response to submersion in heavily aerated seawater (Corpu 1981, 1983) and strip spawning *C. sandwicensis* and *C. talcosa* yields competent larvae in the same time period (CEB, pers. obs.). Recruitment occurs year round, but there are recruitment peaks in January and May for *C. exarata*, one month later in February and June for *C. sandwicensis*, and no recruitment data exists for *C. talcosa* (Kay & Magruder 1977).

To explain the presence of three co-occurring limpets, Kay & Palumbi (1987) and Reeb (1995) proposed that Hawai‘i was colonized on multiple occasions by divergent lineages. Sympatric lineages of *Australium ‘rhodostomum’*, which have a life history similar to *Cellana*, are never sister lineages (Meyer et al. 2005), further suggesting a multiple colonization scenario for Hawaiian...
Cellana. Unlike Australium, where each species pair occupies the same habitat and are morphologically cryptic, the endemic Hawaiian Cellana are readily distinguishable and occupy different habitats (Kay & Magruder 1977). More recently, a population genetic study of the Hawaiian Cellana confirmed that all three Hawaiian species are characterized by a monophyletic mtDNA lineage (Bird et al. 2007). Without nuclear DNA data, however, the genetic confirmation of morphological species designations is incomplete, and without data from additional Cellana species, the origins of the Hawaiian Cellana could not be ascertained. Here we investigate the origins and diversification of the endemic Hawaiian Cellana utilizing broad sampling of Pacific Cellana spp. with three mtDNA loci (12S, 16S, COI; 1565 bp) and two nDNA loci (ATPS; H3; 709 bp). Parallel studies on the distribution and ecology of limpets, primarily by Bird (2006), are the foundations of our interpretation. We subsequently address whether the Hawaiian Cellana meet Coyne & Orr’s (2004) four postulates for sympatric speciation.

Methods

Sample collection, DNA isolation, sequencing, and phasing

In this study, 2274 bp of DNA across five loci from 26 Cellana spp. in the Indo-West Pacific region (including all proximal locations relative to the Hawaiian Islands) were analysed [401 new sequences (GenBank:GQ429294–GQ429326, GQ455807–GQ455986, GQ480460–GQ480477, JF340159–JF340213, JF500635–JF500754) and 497 previously published sequences, all listed in Data S2, Supporting information]. Four limpets from Antarctica, Argentina and Chile in the genus Nacella, which is sister to Cellana (Nakano & Ozawa 2007), served as outgroups. The markers used in this study include mitochondrial 12S (396 bp) (Nakano & Ozawa 2004), 16S (494 bp) (Palumbi 1996), and COI (675 bp) genes (Folmer et al. 1994), as well as the nuclear exon H3 (328 bp) (Colgan et al. 1998) and intron ATPSβ (381 bp) (Jarman et al. 2002).

All Hawaiian islands harboring Cellana, with the exception of Ni’ihau, were surveyed for the presence of C. exarata, C. sandwicensis and C. talcosa. The northwestern Hawaiian Islands were accessed via the National Oceanic and Atmospheric Association Research Vessel Hi’ialakai with permission from the United States Fish and Wildlife Service, the State of Hawai’i Division of Aquatic Resources, and the Papahānaumokuākea Marine National Monument. For tissue sampling, a small piece of foot or mantle tissue (40 mg) was excised using a sterile razor blade and fixed in 95% ethanol. DNA was extracted using DNeasy animal tissue kits (Qiagen). Each DNA fragment was PCR amplified using the primers in Data S1 (Supporting information). PCR reactions were performed using Biomix (Bioline) and prepared for sequencing using Exo-SAP (USB), as detailed in Bird et al. (2007). PCR products were directly sequenced using Big Dye chemistry (Applied Biosystems Inc.) on ABI 3130XL or 3137XL capillary machines at the NSF-EPSCoR sequencing facility at the Hawai’i Institute of Marine Biology and the core sequencing facility at the University of Hawai’i at Mānoa, respectively. All sequences were aligned using ClustalX (Larkin et al. 2007) with default settings and edited by eye.

The nuclear markers, H3 and ATPSβ were directly sequenced and the alleles were inferred (phased) manually using an iterative process (Clark 1990). On the first pass, all homozygous genotypes were set aside as known alleles. On the second pass, all genotypes with a single position ambiguity were split into two known alleles. On the third pass, all genotypes with multiple position ambiguities were matched against known alleles and those that could be described by two known alleles were assigned those alleles. If only one known allele could be present in a genotype, the second allele was inferred and added to the list of known alleles. Electropherograms were double checked to ensure compliance with the known and inferred allelic compositions. Clark (1990) demonstrates theoretically and empirically that incorrect inference of alleles is unlikely when there are no orphaned genotypes (genotypes where neither allele occurs in a homozygote or sequence with a single position ambiguity). There were no orphaned genotypes.

Morphological identification of Hawaiian Cellana

Despite a fair amount of phenotypic plasticity, the three endemic Hawaiian limpets can be readily differentiated by morphological characteristics in the field (Kay & Magruder 1977). C. sandwicensis can be differentiated from C. exarata and C. talcosa by its relatively long mantle tentacles (10–35 mm vs. 2–5 mm), sculptured rugose ridges, scalloped shell perimeter, and elongate shell shape. C. exarata is differentiated by its dark grey to brown to green mantle and smoothed shell ridges relative to C. sandwicensis. C. talcosa has a silver pearlescent nacre on the inside of the shell, no scalloping at the shell fringe, a thick domed shell in large specimens and particularly thin, flat shells in smaller specimens, and a thin solid yellow line with black flecks (relative to a solid black line in other species) at the mantle fringe. All specimens were identified morphologically by CEB using these criteria and others listed by Kay & Magruder (1977) and Powell (1973).
Phylogenetic analysis

Phylogenetic relationships were reconstructed using Bayesian (MrBayes 3.1; (Huelsenbeck & Ronquist 2001) and maximum likelihood methods (RAxML 7.0.4; Stamatakis 2006) on the CIPRES web portal 1.15 (http://www.phylo.org). Gaps were treated as a fifth character state and uncertainties were treated as polymorphisms. Phylogenetic reconstructions were created for each locus individually, as well as with the loci combined. Maximum likelihood (ML) and Bayesian phylogenetic reconstructions for each of the DNA markers, 12S, 16S, COI, and H3 resulted in concordant evolutionary tree topologies, therefore we implemented combined analyses specifying optimized evolutionary models for each of the four markers and only present the ML reconstruction.

Separate analyses were run for ATPSβ because large indels and rearrangements rendered an uncertain alignment for deeper evolutionary separations within Cellana. The ATPSβ sequence data were therefore informative only for closely related species. The only taxa that could be robustly aligned and analysed with the Hawaiian species were C. nigrolineata (Japan), C. grata (Japan and Hong Kong), and C. mazatlandica (Ogasawara), which is wholly consistent with the topol-...
Fig. 2 Maximum likelihood phylogenetic reconstructions for Cellana rooted with Nacella spp. (a) Combined analysis of 12S, 16S, COI, and H3 and (b) separate analysis of ATPSβ alleles for a subset of Cellana spp. were conducted. Branch support values are maximum likelihood bootstrap values and Bayesian posterior probabilities, respectively. Where relevant, Hawaiian Island identities are listed in parentheses and their abbreviations are listed in Fig. 1. Hawaiian Cellana spp. are indicated by black tree branches and bold type.
Based on a full phylogenetic reconstruction using 12S, 16S, COI and H3 and a taxonomically directed reconstruction using ATPSβ, evolutionary monophyly of the endemic Hawaiian Cellana is strongly supported with 93–95% maximum likelihood (ML) bootstrap support and a Bayesian posterior probability of 0.96–1.0 (Fig. 2a,b). Given the extensive coverage of potential sister species (the only missing Pacific taxa are from Fiji and the Juan Fernandez Islands, Powell 1973; Nakano & Ozawa 2004, 2007; Nakano et al. 2009), all phylogenetic analyses indicate that the sister group to Hawaiian limpets is composed of C. grata (Japan and China) and C. mazatlantica (Ogasawara) (Fig. 2).

The monophyly of each Hawaiian species (C. exarata, C. sandwicensis and C. talcosa) is robustly supported with 100% ML bootstrap support and Bayesian posterior probabilities of 1.0 in both the 12S, 16S, COI, and H3 multimarker phylogeny and the ATPSβ phylogeny (Fig. 2), with the exception of C. talcosa in the ATPSβ phylogeny, which has bootstrap support of 80% and a posterior probability of 1.0. The cladograms and parsimony networks for COI, ATPSβ, and H3 (Fig. 3) also exhibit three distinct lineages of Hawaiian Cellana, with the exception of the slowly evolving nuclear exon, H3, where C. talcosa is fixed for a single allele that is shared with C. sandwicensis – consistent with incomplete lineage sorting. In addition, the taxa in Fig. 3 form a polytomy in the phylogeny of H3. For the ATPSβ intron (Fig. 3), C. sandwicensis and C. talcosa cannot be separated into individual networks because they form a paraphyletic lineage. In the ATPSβ phylogeny (Fig. 2), four C. sandwicensis alleles are basally rooted with marginal branch support (bootstrap of 48% and posterior probability of 0.79) relative to the other alleles of C. sandwicensis and C. talcosa, exhibiting detectable hierarchy within a species (unlike H3). In the more rapidly evolving COI, each taxonomic network is separated into monophyletic clades (Fig. 3). Using the phylogeny for COI, a basally rooted haplotype can be identified for C. talcosa, and can be used to polarize the haplotype network (denoted by a black square in Fig. 3). Notably, C. talcosa exhibits a shallow mtDNA partition between Kaua‘i and the other Main Hawaiian Islands (bootstrap = 89%, posterior probability = 0.97, Figs 2a and 3), and the COI haplotype network indicates that the Kaua‘i mtDNA lineage is young relative to that in the other Main Hawaiian Islands (Fig. 3) despite being the oldest sampled island on which C. talcosa is found (Fig. 1).

**Reproductive isolation among Hawaiian Cellana**

In the examination of 81 C. exarata, 84 C. sandwicensis, and 103 C. talcosa from the Main Hawaiian Islands where all three species co-occur, evidence of hybridization was detected between C. sandwicensis and C. talcosa and between C. exarata and C. talcosa (Table 1). Specifically, two individuals (Moloka‘i and Kaua‘i) were morphologically identified as C. sandwicensis, have the mtDNA ATPSβ alleles of C. sandwicensis, but have the mtDNA COI haplotypes of C. talcosa. The potential introgression of C. talcosa mtDNA into C. sandwicensis can be visualized in the COI haplotype network of C. talcosa (Fig. 3). With only one nuclear locus (H3 is not informative for discrimination between C. sandwicensis and C. talcosa), we cannot conclude whether this is evidence of recent hybridization or older introgression, but we suspect introgression because the morphology and ATPSβ alleles both indicate the two individuals are C. sandwicensis. A single individual was observed with the mtDNA of C. talcosa, one H3 and ATPSβ allele of C. talcosa, and one H3 and ATPSβ allele of C. exarata, indicating a potential F1 hybrid (see striped ATPSβ alleles in Fig. 3, the H3 alleles in the hybrid are not marked). Overall, the mean estimated occurrence of C. sandwicensis-C. talcosa putative introgression is 0.94% (±1.1%, 95% confidence interval) and the mean estimated occurrence of C. exarata-C. talcosa putative F1 hybrids is 0.45% (±0.90%, 95% confidence interval). We conclude that hybridization is rare and that the three species are reproductively isolated.

**Molecular dating**

A relaxed molecular clock model run in BEAST places the colonization of Hawai‘i by Cellana at ~5.1 Ma (3.4–7.2 Ma HPD interval), approximately the same age as the oldest contemporary basaltic islands occupied by all three species, Ni‘ihau and Kaua‘i (Fig. 1). The C. exarata lineage diverges from that of C. sandwicensis and C. talcosa ~4.3 Ma (2.6–6.1 Ma HPD interval) and the C. sandwicensis and C. talcosa lineages diverge ~2.3 Ma (1.2–3.4 Ma HPD interval), mirroring the vertical stratification of the species from the high to low shore.

**Ancestral character state**

The closest extant relatives of the original Hawaiian colonists, Cellana nigrolineata (Japan), C. grata (Japan and China) and C. mazatlantica (Ogasawara), are all specialist high-shore limpets (Fig. 3) (Fukuda 1993; Williams & Morritt 1995; Kubota & Torigoe 2000). Thus, we propose that high shore habitat is an ancestral character state for the Hawaiian Cellana, a state exhibited by the widespread and basally rooted Hawaiian limpet, C. exarata.

**Discussion**

The archipelagos which stretch across the tropical Pacific Ocean are excellent natural laboratories in which to
investigate marine speciation (Paulay & Meyer 2002). The Hawaiian Islands are among the most isolated of these archipelagos and have been intensively studied. Many terrestrial organisms have colonized the Hawaiian archipelago and subsequently diversified in circumstances with plausible allopatric scenarios (Cowie & Holland 2008), including the *Argyroxiphium* plants (Baldwin & Robichaux 1995), *Succineid* land snails (Holland & Hadfield 2004; Rundell et al. 2004), *Drosophila* fruit flies (Carson & Kaneshiro 1976; O’Grady & DeSalle 2004), *Hyposmocoma* moths (Rubinoff 2008), *Mecaphesa* and *Theridion* spiders (Gillespie 2004; Garb & Gillespie 2009), and *Drepanidinae* honeycreeper birds (Tarr & Fleischer 1995). However, as noted by Kay & Palumbi (1987) ‘…the marine fauna of the Hawaiian Islands is differentiated from its Indo-West Pacific roots but has not diversified.’ Indeed, there are few known cases of diversification among insular marine organisms (Hellberg 1998; Cunha et al. 2005; Dawson & Hamner 2005; Rocha et al. 2005; Faucci et al. 2007) and no known cases of broadcast-spawners diversifying within archipelagos, only among archipelagos (Meyer et al. 2005).

In the terrestrial Hawaiian radiations, there is often a pattern of phylogenetic partitioning by isolated regions within an island, as well as a pattern of younger lineages on younger islands – a phenomenon widely known as the progression rule (Funk & Wagner 1995). The cone snails of the Cape Verde islands (*Conus*) exhibit a striking pattern of complete concordance of geographic location and genetic identity, even within islands, which is likely facilitated by internal fertilization and crawl-away larvae (Cunha et al. 2005). The *Mastigias* jellyfish of Palau (Dawson & Hamner 2005) and *Halocaridina rubra* anchialine shrimp of Hawai‘i (Santos 2006; Craft et al. 2008) exhibit a similar pattern of genetic distinctiveness in land-locked marine lakes and ponds. Consistent with the conclusion of Kay & Palumbi (1987), the broadcast-spawning turbinid snails, *Astralium ‘rhodostomum’*, differentiate after colonizing an archipelago but do not diversify within the archipelago (Meyer et al. 2005). The phylogenetic relationships between putative species in the *Astralium ‘rhodostomum’* complex exhibit a distinct signature of allopatric speciation because sympatric taxa are not sister taxa (Meyer et al. 2005). Based on these cases, one would expect...
to see a genetic signature of allopatric speciation in closely related species within and among the Pacific archipelagos.

The evolutionary history of limpets in Hawai‘i can be summarized as a rare colonization event ~5 Ma, followed by diversification along an ecological gradient within the basaltic littoral zone of the Hawaiian Islands. Given the geological history of the Hawaiian Islands in which volcanic islands rise thousands of meters from the sea floor, all biota originally arrived by dispersal, and researchers have debated the colonization routes into Hawai‘i for decades. For marine species, the predominant possibilities may be summarized as colonization from the West Pacific, possibly via the warm Kuroshio Current (Hourigan & Reese 1987) or colonization from the South Pacific, possibly via the trans-equatorial Line Islands and Johnston Atoll (Gosline 1955). The former is supported by the presence of some West Pacific fishes exclusively in the most northwestern islands of Hawai‘i (beyond those displayed in Fig. 1), including the Japanese angelfish (*Centropyge interrumpia*) and the blotcheye squirrelfish (*Myripristis murdan*) (Pyle 1999; Mundy 2004). The latter is supported by the distribution of the flame angelfish (*Centropyge loriculus*) and the chevron butterflyfish (*Chaetodon trifascialis*) (Mundy 2004). In a survey of endemic butterflyfishes of Hawai‘i, two of the three species had closest relatives in the West Pacific (Craig et al. 2010). The *Cellana* add the first marine invertebrate case of colonization from the West Pacific to Hawai‘i, and reinforce the importance of that pathway for Hawaiian marine biodiversity.

The diversification of limpets in Hawai‘i occurred without absolute gene flow barriers to induce evolutionary-scale isolation. Although Hawaiian limpets exhibit significant population structure indicating gene flow restrictions and semi-permeable barriers among some populations (Bird et al. 2007), our survey did not reveal any allopatric separations that can be interpreted as proof of incipient speciation within contemporary limpets. With impermeable geographic barriers to gene flow lacking, we must consider that natural selection could have driven reproductive isolation in this diversification of limpets into distinct habitats. Ernst Mayr (1963) defined sympatric speciation as that which occurs within the cruising range of an organism. We take this to mean speciation within the range over which a typical individual is expected to travel within its lifetime, which can be quite large in species with pelagic larvae. On a fine (micro) spatial scale, the gametes of wave-dominated intertidal broadcast-spawners such as the Hawaiian *Cellana* are sympatric in the well-mixed very near shore water column. Consequently, broadcast-spawning marine species with pelagic larvae are considered unlikely candidates to radiate within an archipelago (see Teske et al. 2007), prompting Coyne & Orr (2004) to identify such cases as the ‘acid test’ for speciation in sympatry. The Hawaiian *Cellana* clearly exhibit three of the four characters that Coyne & Orr (2004) identify as indicative of sympatric speciation: sympatric species distributions (Fig. 1), a monophyletic evolutionary lineage (Fig. 2), and reproductive isolation (Fig. 3, Table 1).

**Monophyly: Colonization and species diversification within the Hawaiian archipelago**

The monophyletic lineage of Hawaiian *Cellana* indicates a single colonization of Hawai‘i from the China/Japan/Ogasawara region (>4200 km). The alternative conclusion of multiple colonizations of Hawai‘i is less parsimonious, given the phylogenetic reconstructions (Fig. 2), because it requires additional unsupported assumptions: (i) multiple traverses of thousands of km...
of open ocean and (ii) the extinction of the source population(s). Given that Hawai‘i is the most isolated archipelago in the Pacific and no basaltic islands have existed between Japan and Hawai‘i in the past 30 My, no obvious ‘stepping stone’ candidates exist (Vermeij 1971; Kay & Magruder 1977; Williams & Morritt 1995). If we assume a maximum larval duration of three weeks, the lecithotrophic larvae would have to average 8.3 km/h to travel from either Japan or Ogasawara to Hawai‘i, which is unlikely. Rather, it is more plausible that Cellana rafted to Hawai‘i on a log or pumice stone. Kay and Magruder (1977) document Cellana inhabiting the trunks of palm trees residing on a plateau that slid into the ocean, thereby demonstrating a feasible rafting mechanism in the geologically active region where Hawaiian Cellana originated.

When considering the molecular phylogeny (Fig. 1), the vertical stratification of limpet habitats in the intertidal zone, and the outgroup trait mapping of the ancestral character state (Fig. 3), we propose that the high shore was occupied by the original colonizing Cellana, the mid-low shore was colonized second by the C. sandwicensis/talcosa lineage (~4.3 Ma), and the low/subtidal shore was colonized third by the C. talcosa lineage (~2.3 Ma). The high intertidal zone requires adaptations to thermal stress and desiccation that most marine species lack (Somero 2002), but high shore species often exhibit lower fitness when experimentally moved into the ‘less stressful’ low shore environment due to poor competitive ability and predation (Connell 1961a,b; Paine 1974). It is most parsimonious to assume that a high shore species could more readily survive and adapt to the mid-low littoral shore than the subtidal environment, prompting our conclusion of colonization from high to middle to subtidal habitats over evolutionary time.

Reproductive isolation among Hawaiian Cellana

Based on available data, rare hybridization/introgression has occurred among some of the extant lineages of Cellana in Hawai‘i. A low level (0.9%) of putative mitochondrial introgression is evident between C. sandwicensis and C. talcosa (Table 1, Fig. 3), demonstrating that hybrids have successfully back-crossed. We additionally observed a single putative FI hybrid between C. exarata and C. talcosa, where back-crossing is not evident. Overall, the Hawaiian Cellana remain morphologically distinct (Kay & Magruder 1977), each is characterized by monophyletic mitochondrial lineages (12S, 16S, COI), and C. exarata and C. talcosa are characterized by monophyletic nuclear lineages (ATPS8) thereby verifying their reproductive isolation, and indicating that the species are diverging rather than merging. These data can have three explanations which are not mutually exclusive: (i) the species are mostly reproductively incompatible, (ii) ecological and environmental factors are limiting the opportunities for hybridization, and (iii) selection reduces the fitness of hybrids. Further research is required to determine which of these hypotheses are supported.

Likelihood of allopatric speciation

All pertinent evidence indicates that the Hawaiian Cellana are sympatric monophyletic lineages with reproductive isolation. However, assessing whether Hawai‘i constitutes ‘an evolutionary and geographic setting where allopatric speciation is improbable’ (sensu Coyne & Orr 2004) is subjective. One of the major obstacles in studying speciation mechanisms in natural populations is that our observations are merely snapshots of the speciation process. Littorina saxatilis is considered to be in the beginning stages of sympatric speciation (Roland-Alvarez 2007), but it is unknown if the process will proceed to completion. The Hawaiian Cellana nicely compliment the example of L. saxatilis because they represent the other end of the spectrum where reproductive isolation is nearly complete but, unlike L. saxatilis, with Cellana we cannot observe whether the lineages were sympatrically distributed when they first diverged. Thus, we are left to assess the circumstantial evidence.

Unlike previously reported cases of diversification within an archipelago, such as the Cape Verde Conus snails (Cunha et al. 2005, 2008), the Palauan land-locked Mastigias jellyfish (Dawson & Hamner 2005), or many of the various Hawaiian terrestrial radiations, there is no clear phyleogeographic signature of allopatric speciation in the Hawaiian Cellana. The phyleogeography of Hawaiian Cellana does not follow the progression rule (Funk & Wagner 1995), where older lineages reside on older islands. Rather the ancestral lineage of Hawaiian Cellana has the greatest range, which overlaps 100% with the ranges of the derived lineages (Fig. 1) – not a signature of allopatric speciation. The fact that the Hawaiian Cellana are sympatrically distributed differentiates them from the cases of the allopatrically distributed Cape Verde cone snails and Palauan jellyfish. The only phyleogeographic pattern evident in the Hawaiian Cellana is observed in C. talcosa (putatively most derived character state for low shoreline habitat), where Kaua‘i shares no COI haplotypes with the other Main Hawaiian Islands (Figs. 2 and 3) (Bird et al. 2007). This pattern is not evident in the more slowly mutating nuclear DNA (Fig. 3), indicating a recent population-level partition rather than an evolutionary divergence. Across this partition, the \( N_M \) is estimated to be as high as 0.16.
using a coalescent-based analysis (Bird et al. 2007) – too high for reproductive incompatibility to develop from isolation alone. Regarding the other two species, the most abundant COI haplotype in C. exarata and the second most abundant haplotype in C. sandwicensis occur on every island investigated across their entire species ranges (Bird et al. 2007). For C. exarata, N, M is estimated to be as high as 0.77 between the Northwestern Hawaiian Islands (NWHI) and the main Hawaiian Islands (MHI), 9.0 within the MHI, and 8.1 among the Hawaiian Islands (NWHI and the main Hawaiian Islands (MHI), 9.0 within the MHI, and 0.81 among the NWHI (Bird et al. 2007). For C. sandwicensis, N, M is estimated to be as high as 11.0 between the NWHI and MHI, 55.0 within the MHI, and 4.3 within the NWHI. Again, there is no genetic signature of long-term isolation resulting in allopatric speciation.

In cases such as the Hawaiian Cellana where species are sympatric and impermeable geographic barriers to gene flow are absent, the phenomenon of transient allopatry has been proposed as a potential explanation (Hellberg 1998; Kelly & Eernisse 2008). There are gene flow restrictions between populations of each Hawaiian Cellana spp. thus transient allopatry cannot be ruled out, but a specific mechanism is lacking. All the inferred gene flow restrictions among Hawaiian Cellana populations are aligned with channels between islands, which are currently at their maximum width because interglacial sea level is currently high (see Clague 1996; Neall & Trewick 2008). In fact, 56–87% of the variation in genetic differentiation (FST) among population samples of Hawaiian Cellana is attributable to distance where increasing channel width is related to increased genetic differentiation (Bird et al. 2007). Therefore, past (glacial) drops in sea level would only serve to slightly narrow the deep channels, reveal currently submerged habitat (Fig. 1), and reduce genetic differentiation among limpets on different islands. Other sympatric assemblages of marine species may be plausibly explained by transient allopatry, but there is no parallel explanation available for the Hawaiian Cellana.

**Likelihood of ecological and sympatric speciation**

Ecological factors have been increasingly implicated as contributors to marine biodiversity and speciation (Hellberg 1998; Fauchi et al. 2007; Rolan-Alvarez 2007; Rocha et al. 2008). The steep gradient of thermal, hydrodynamic, and desiccation stress across the intertidal shoreline habitat (Bird 2006) causes biotic zonation patterns world-wide (Stephenson & Stephenson 1972), and provides ample opportunity for disruptive ecological selection. The intertidal snail L. saxatilis seems to be in the early stages of this process, wherein selective pressures are driving partial reproductive isolation among vertically stratified morphotypes (Quesada et al. 2007; Rolan-Alvarez 2007). Likewise, Hawaiian Cellana are vertically stratified across an intertidal ecotone, indicating that ecological selection has played a role in their diversification; otherwise, we might expect to see a cryptic species complex without discrete habitat affinities, as is the case with the Astralium ‘rhodostomum’ turbinid snail complex (Meyer et al. 2005). In any case, speciation along geographic and ecological boundaries are not mutually exclusive, and can work in concert (Nosil 2008a; Schluter 2009).

A plausible non-geographic mechanism for the diversification of the Hawaiian Cellana species residing at different intertidal levels, consistent with the data at hand, involves divergent selection (Rice & Pfennig 2010) and allochronic assortative mating (timing of gamete release) (Jankowski & Straile 2004; Savolainen et al. 2006; Friesen et al. 2007). As the original high shore colonists expanded into the lower portions of the littoral zone, they encountered strong selective pressures in the novel habitats which can drive genetic divergence (Mallet et al. 2009; Rice & Pfennig 2010). Low shore offspring are expected to have lower fitness on the high shore and vice versa, and hybrids are expected to have lower fitness thereby driving lineage diversification via disruptive selection. Lower shore survivors are more likely to reproduce with each other due to synchronous spawning, which is common in broadcast-spawners (see Levitan et al. 2004), and pass on advantageous characters to their offspring. Further, the spawning cue for the high-shore C. exarata involves immersion in heavily aerated water on the new moon (Corpuz 1983), so that lower shore limpets would be expected to spawn prior to those on the higher shore or rely upon a different cue. Different spawning cues would decrease cross-fertilization and the proportion of hybrid offspring, as is found in the Howea palms (Savolainen et al. 2006). Although gene flow among limpets at different shore elevations may not cease completely, recent studies demonstrate that natural selection can drive population differentiation to complete speciation in spite of ongoing gene flow (Nosil 2008a; b). The diversification scenario proposed here for Hawaiian Cellana is consistent with available evidence and nearly identical to that for the Howea palms. As with the intertidal marine snail L. saxatilis (Johannesson et al. 2010), the marine reef fishes Hexagrammos otakii and H. agranum (Crow et al. 2010), the Mediterranean algae Fucus radicans and F. vesiculosus (Forslund & Kautsky 2010), and the North Atlantic killer whales Orcinus orca (Foote et al. 2009) we cannot reject an allopatric hypothesis, but sympatric speciation along ecological boundaries is our favored explanation for the Hawaiian limpet radiation.
Conclusions
The difficulty in reconciling the great diversity of marine life with their considerable dispersal potential demonstrates that much remains to be learned about speciation in the sea. Prior to this report, no marine species were known to have diversified in Hawai‘i and no broadcast-spawners were known to have differentiated within archipelagos, but we suspect that more cases will emerge. It is apparent that the three Hawaiian Cellana form a closely related monophyletic lineage that has speciated within the Hawaiian archipelago. The fact that these limpets have mildly overlapping vertical distributions, release gametes into the water prior to fertilization, leave mate choice to sperm-egg recognition proteins, and have mobile pelagic larvae capable of persisting for over two weeks makes them unlikely candidates for diversification. However, the segregation of ‘ōpūhi species into vertically stratified zones along an obvious selection gradient, the littoral zone, is evidence that selection for ecotypes and adaptive diversification has occurred. Together, these findings support the hypotheses that speciation along ecological gradients contributes to marine diversity, marine speciation does not require as much geographic segregation as once thought, and complete allopatric isolation may not be necessary to drive diversification of marine lineages.

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References


C.E.B. studies coastal molecular community ecology and believes in the sanctity of ‘ōpūhi. B.S.H. is a gentleman with diverse molluscan interests. B.W.B. studies the phylogeography of marine vertebrates but acknowledges lessons from the other ~32 phyla in the animal kingdom. R.J.T. studies marine invertebrates but also acknowledges lessons from the chordata.

Data Accessibility


Supporting information

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

Data S1 PCR primers used in this study.

Data S2 Samples used in this study with GenBank accession numbers.

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