Molecular Ecology (2007) **16**, 2422–2435 doi: 10.1111/j.1365-294X.2007.03246.x

A geographic mosaic of passive dispersal: population structure in the endemic Hawaiian amber snail *Succinea caduca* (Mighels, 1845)

BRENDEN S. HOLLAND and ROBERT H. COWIE
Center for Conservation Research and Training, Pacific Biosciences Research Center, University of Hawaii, 3050 Maile Way, Gilmore 408, Honolulu, Hawaii 96822, USA

Abstract

We used 276 cytochrome *c* oxidase subunit I (COI, 645 bp) and a subset of 84 16S large ribosomal subunit (16S, 451 bp) sequences to evaluate geographic patterns of genetic variation in 24 populations of the endemic Hawaiian land snail *Succinea caduca* spanning its range on six islands. Haplotype networks, gene tree topologies, pairwise molecular divergence and F_{ST} matrices suggest substantial geographic genetic structuring and complex dispersal patterns. Low nucleotide diversity and low pairwise molecular divergence values within populations coupled with higher between population values suggest multiple founder events. High overall haplotype diversity suggests diversification involving rare interpopulation dispersal, fragmentation by historical lava flows and variation in habitat structure. Within-island rather than between-island population comparisons accounted for the majority of molecular variance. Although 98% of 153 COI haplotypes were private by population, a Mantel test showed no evidence for isolation by distance. Mismatch distributions and population partitioning patterns suggest that genetic fragmentation has been driven by punctuated, passive dispersal of groups of closely related haplotypes that subsequently expanded and persisted in isolation for long periods (average > 2 million years ago), and that Pleistocene island connections may have been important in enhancing gene flow. Historical availability of mesic coastal habitat, together with effective dispersal may explain the long-term persistence and unusual multi-island distribution of this species, contrasting with the single-island endemism of much of the Hawaiian biota.

Keywords: founder events, Hawaiian Islands, island phylogeography, mtDNA, Pleistocene land bridge, Succineidae

Received 23 September 2006; revision accepted 20 November 2006

Introduction

Oceanic islands, such as the Azores, Canaries, Marquesas and notably the Hawaiian islands, are home to unique forms of endemic plants and animals that evolved in isolation over millions of years. The extreme insularity, diversity of microhabitats and dynamic geology and age structure of the Hawaiian Islands are the principal attributes leading to their unparalleled biological diversity and endemism (e.g. Simon 1987). Hawaii's unique terrestrial radiations have attracted scientific attention for over a century (Gulick 1905; Carson 1987; Wagner & Funk 1995; Hormiga *et al*. 2003). In recent decades, however, the Hawaiian biota has become increasingly threatened. Extensive anthropogenic habitat alteration and introduction of thousands of non-native species (Eldredge & Evenhuis 2003; Pimentel *et al*. 2005) have led to high levels of extinction among the native flora and fauna (e.g. Vitousek 1988; Pimm *et al*. 1994; Wagner *et al*. 1999).

Endemic land snails constitute some of the major terrestrial radiations in the Hawaiian Islands and present excellent opportunities for the study of historical biogeography and evolutionary diversification of insular lineages. Hawaiian land snail diversity includes about 750 described species, comparable to the diversity of the North American land snail fauna (Pilsbry 1938–1948), with over 99% endemism (Cowie 1996). However, losses of land snail species

Correspondence: Brenden S. Holland, Fax: (808) 956-2647; E-mail: bholland@hawaii.edu

have been estimated as 65–75% (Solem 1990) or perhaps as high as 90% (Cowie 2001). Among the remaining extant snail species, including some of the best studied groups such as achatinelline tree snails, range reductions have been estimated at up to 90% (USFWS 1993). With these pressing conservation concerns as a backdrop, we seek to elucidate natural patterns of species diversification and historical biogeography in the Hawaiian Islands, with a focus on the land snail fauna.

Information regarding patterns of dispersal and population structure of endemic land snails in the Hawaiian Islands is important not only for understanding their origins, both geographic and phylogenetic, but also for illuminating general models of evolutionary diversification and developing effective conservation strategies. In spite of the imperilled nature of Pacific island land snail fauna, very few studies of molecular population structure have been published (Murray *et al*. 1991; Goodacre 2002; Holland & Hadfield 2002).

The Succineidae are adept at dispersing across vast geographic distances, as evidenced by their worldwide distribution that includes most Pacific islands. In Hawaii, the 42 described endemic species (Cowie 1996) have radiated into a wide variety of habitats on all the main islands, from montane dry scrubland to cloud forests and xeric coastal duneland. Perhaps in part because of their relatively high growth rates and fecundity (Rundell & Cowie 2003) and diverse habitat preferences, some succineid species have fared relatively well compared to other Hawaiian land snails in the face of habitat loss and the impacts of invasive species (Hadfield *et al*. 1993). The Hawaiian Succineidae thus provide an informative group for testing evolutionary and biogeographic hypotheses using molecular techniques.

Our approach utilized COI and 16S mitochondrial DNA (mtDNA) markers to investigate population structure within and among the Hawaiian Islands. Although a number of recent criticisms of the use of mtDNA markers for inferring evolutionary history have been published (e.g. Shaw 2002), nuclear genes also suffer from various technical drawbacks, chief among them for population genetic analysis is their lack of within-species sequence-based polymorphism, due to low substitution rates (see Rubinoff & Holland 2005).

Taxa lacking highly dispersive life histories, such as crickets (Shaw 2002), drosophilids (DeSalle 1995) and certain plants (Funk & Wagner 1995), tend to have radiations characterized by single-island or single-volcano endemism (Roderick & Gillespie 1998; Gillespie & Roderick 2002), as do nearly 90% of the endemic Hawaiian land snail species (Cowie 1996). Such species are not as useful as species that occur on multiple islands for testing phylogeographic hypotheses of how populations have achieved their present distributions. Only one species of endemic Hawaiian land snail, *Succinea caduca* Mighels, 1845, is distributed on all of the main islands: Kauai, Oahu, Molokai, Lanai, Maui and Hawaii (Holland & Cowie 2006). *S. caduca* inhabits both pristine and highly modified xeric coastal and lowland habitats. This study focuses on the phylogeography and molecular population structure of *S. caduca* and represents the most comprehensive sampling and molecular analysis of a single Hawaiian species. Our objectives were to: (i) confirm the monophyly of snails putatively identified as *S. caduca* from all six main islands; (ii) assess the phylogeographic relationships and evaluate population genetic partitioning within and among islands; (iii) develop a hypothesis for the mechanisms of diversification of *S. caduca* populations in time and space; (iv) estimate the age of the lineage (presuming demonstration of monophyly) in relation to the geological ages of the Hawaiian Islands; (v) evaluate the importance of Pleistocene land bridges and historical island habitat availability in maintaining interisland gene flow; and (vi) provide a preliminary conservation genetic assessment of this species.

Materials and methods

Sampling

In total, 276 *Succinea caduca* were collected from 24 sites on six islands spanning nearly 500 km (Fig. 1). More populations were found on Oahu (12) than on other islands despite extensive surveys on those islands. Snails were collected primarily during the rainy season and mainly during or within a few days of heavy rainfall. The Hawaiian climate is dominated by the northeast trade winds, which deliver moisture mostly over windward slopes (Carson & Kaneshiro 1976). With a few exceptions (populations I, O, P, Q, S; Fig. 1), the majority of collection sites were in leeward coastal areas that are relatively dry (Fig. 1), although even the windward sites were in areas of relatively lower rainfall. For additional details of collection sites see Holland & Cowie (2006). Active *S. caduca* were encountered only when heavy sustained precipitation had resulted in standing water, flowing runoff and mats of cyanobacteria and subaerial algae. At other times, these small (maximum shell length ~8 mm), cryptic snails were located following careful searching of the undersides of lava boulders, shaded overhangs and consolidated volcanic ash crevices, among leaf litter, and at the bases of dense clumps of grass, in some cases as close as 10 m from the beach.

DNA extraction, PCR amplification and sequencing

Molecular sequence data were obtained from all 276 specimens for COI (645 bp) and a subsample of 84 specimens for 16S (451 bp). Fragments of the COI and 16S genes were analysed both together and separately to infer intraspecific relationships among populations from the six islands.

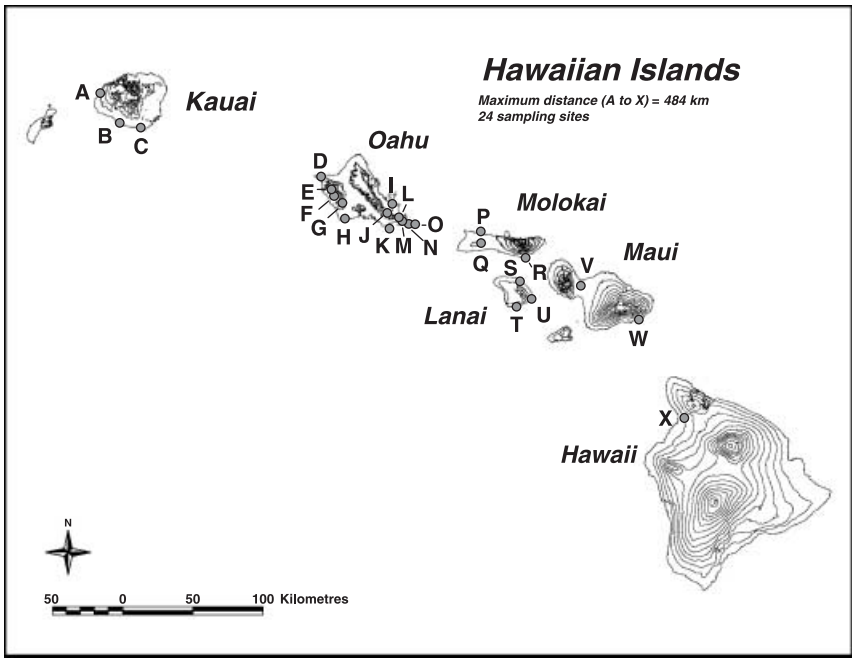

Fig. 1 Map of Hawaiian Islands showing sampling localities for *Succinea caduca*. Locality details are the following: A, Barking Sands (22°00′42″N, 159°46′21″W, Kauai); B, Russian Fort (21°56′89″N, 159°40′25″W, Kauai); C, Port Allen (21°53′83″N, 159°34′98″W, Kauai); D, Kaena Point (21°34′41″N, 158°15′20″W, Oahu); E, Makua Valley (21°32′00″N, 158°14′33″W, Oahu); F, Makaha Valley (21°28′53″N, 158°13′24″W, Oahu); G, Lualualei Valley (21°24′81″N, 158°08′32″W, Oahu); H, Barbers Point (21°17′70″N, 158°05′00″W, Oahu); I, Kailua Puu O Ehu (21°23′40″N, 157°45′12″W, Oahu); J, Waahila Ridge (21°18′30″N, 157°48′61″W, Oahu); K, Diamondhead Crater (21°15′50″N, 157°48′60″W, Oahu); L, Wilhelmina Rise (21°17′00″N, 157°57′40″W, Oahu); M, Kalanianaole Highway (21°16′63″N, 157°41′80″W, Oahu); N, Koko Crater (21°17′12″N, 157°40′70″W, Oahu); O, Makapuu Point (21°18′54″N, 157°39′20″W, Oahu); P, Moomomi Preserve (21°11′95″N, 157°09′87″W, Molokai); Q, Highway 460 Maunaloa (21°08′80″N, 157°11′70″W, Molokai); R, Highway 450 Kamalo (21°03′10″N, 156°53′31″W, Molokai); S, Kahokunui (21°53′48″N, 156°53′90″W, Lanai); T, Kaumalapau (20°46′95″N, 156°58′70″W, Lanai); U, Manele Bay (20°44′73″N, 156°53′51″W, Lanai); V, Waikapu (20°49′19″N, 156°31′08″W, Maui); W, Apole Point (20°37′75″N, 156°10′34″W, Maui); X, Makeahua Gulch (20°01′76″N, 155°49′35″W, Hawaii).

Genomic DNA were extracted according to the manufacturer's protocol using QIAGEN DNeasy nucleic acid extraction kits. DNA were eluted in de-ionized autoclaved water, and stored at –80 °C. Universal COI (Folmer *et al*. 1994) and 16S (Garey *et al*. 1998) polymerase chain reaction (PCR) oligonucleotide primers were used to amplify target fragments using a PTC-200 thermocycler (MJ Research). Primer sequences for COI were LCO1490 (5′-GGTCA-ACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGATGACCAAAAAATCA-3′) and for 16S were 16S1 (5′-CGCCTGTTTATC AAAAACAT-3′) and 16S2 (5′-CTCCGGTTTGAACTCAGATC-3′). COI and 16S PCR conditions were identical, as follows: 2 min at 92 °C, 35 cycles of 94 °C for 30 s, 48 °C for 30 s and 72 °C for 45 s, with a final 72 °C extension for 7 min.

PCR-amplified DNA fragments were purified with QIAquick· spin columns manufactured by QIAGEN, according to the manufacturer's protocol, and quantified via agarose minigel electrophoresis. Amplified mtDNA strands were cycle-sequenced using PCR primers. Sequences were determined using the commercial service Macrogen. COI sequences were aligned by eye; 16S sequences were aligned with CLUSTAL X 1.83.1 (Thompson *et al*. 1997).

Population structure analysis and gene flow estimates

We computed comparative statistics for DNA sequences using several methods to quantify and understand the distribution of polymorphic sites and to infer patterns of gene flow vs. reproductive isolation. DNA divergence among populations was estimated assuming the island model of population structure using N_m (Nei 1982) and F_{ST} (Hudson *et al*. 1992). Population structure was examined primarily among populations within islands and among islands with populations from each island grouped together. Although data were generated for COI and 16S mtDNA gene fragments, for most populations from all six islands the substitution rate is higher for COI making it the more appropriate and informative population level marker. Therefore, COI data were the focus of population structure analyses.

A coalescent estimation approach was employed to compare fixation indices F_{ST} among and within-population

groupings, using DNASP (Rozas *et al*. 2003). DNASP was also used to estimate population-level parameters and their variances, including the coefficient of gene differentiation G_{ST} (Nei 1982), pairwise nucleotide diversity π, haplotype diversity *h*, mean number of haplotype substitutions per site between populations D_{xy}, number of net nucleotide substitutions per site and number of migrants N_m (Hudson *et al*. 1992).

Traditional population differentiation indices (F_{ST}) rely on a number of assumptions, including mutation-drift equilibrium and selective neutrality of the markers used. We tested for departures from equilibrium in each population with Tajima's *D* statistic (Tajima 1989), Fu and Li's *F** and *D** (Fu & Li 1993), and by plotting the frequency distribution of pairwise differences in mtDNA sequences as proposed by Slatkin & Hudson (1991) and Rogers & Harpending (1992). A mismatch distribution plot was produced for each 23 of the 24 populations (not V, only one snail sampled). Tajima's *D* contrasts estimates of the population mutation parameter θ, based on π, with those based on the number of segregating sites, for a given sample size. This test statistic is sensitive to demographic effects such as changes in population size. Fu & Li's (1993) statistics contrast estimates of θ based on mutations in internal vs. external branches of the tree. Designed to assess neutrality, the tests assume that more recent mutations occur near the tips of branches, while older substitutions are internal, and recent mutations conferring a selective advantage would increase to high frequency rapidly. Selection results in an excess of identical haplotypes, or mutations in the external branches, and negative values of *D** and *F** (Fu & Li 1993). As with Tajima's *D*, recent population expansions can also result in an excess of external substitutions and negative values of test statistics.

The program MEGA (Kumar *et al*. 2004) was used to generate pairwise molecular divergence matrices and standard errors using the Kimura 2-parameter (K2P) substitution model to correct for a higher probability of transitions than transversions.

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 by island, using ARLEQUIN 3.01 (Excoffier *et al*. 2005). We used AMOVA to estimate variance components and *F*-statistic analogues (*F*-statistics), reflecting the correlation of haplotype diversity at different levels of hierarchical subdivision. Significance of Φ-statistics was tested by 1000 permutations of haplotypes among and within populations under the null hypothesis of panmixia. Significance of variance components was also tested using a permutational approach. Nei's average pairwise genetic distance and exact tests of population differentiation were also computed with ARLEQUIN. Tests based on the infinite-alleles (Slatkin 1995) and infinite-sites models (Tajima 1989; Fu 1997) were used to test for significant deviations in haplotype frequencies from the neutral expectation (Slatkin & Barton 1989) as implemented in ARLEQUIN.

Statistical parsimony networks were constructed with TCS version 1.18.mac (Clement *et al*. 2000) for the COI data set. TCS was implemented with a 95% connection limit.

Isolation-by-distance analysis

The term 'isolation by distance' (IBD) is used to describe a pattern of population genetic variation that derives from spatially limited gene flow. This population genetic model is defined as a decrease in the genetic similarity between populations as the geographic distance between them increases. Statistical tests for IBD were conducted using the 24 populations as units of replication with mtDNA sequences grouped into populations. A nonparametric Mantel test was performed to test for nonrandom associations between a matrix of corrected genetic distances between all population pairs and a matrix of pairwise geographic distances, using the program IBD (Jensen *et al*. 2005) with 30 000 randomizations and a major axis regression.

Phylogenetic reconstruction

Phylograms were generated for COI alone and for 16S and COI together using maximum parsimony (MP), minimum evolution (ME) and maximum likelihood (ML), via heuristic searches in PAUP* 4.10b (Swofford 2002). MP reconstructions were run using default settings. Distance-based reconstructions were carried out using ME and corrected via the K3P model in light of empirically determined biases in terms of transition/transversion ratios and third position substitutions. The ML reconstruction was run with user-defined parameters and HKY+I+G, generated by MODELTEST 3.06 (Posada & Crandall 1998) and node support values were generated based on 1000 bootstrap replicates.

Results

Population structure

Population structure for 276 COI sequences (GenBank Accession nos DQ658422–DQ658684, AY150081) from 24 populations on six islands (Fig. 1) was assessed by various means, including haplotype diversity (*h*) and nucleotide diversity (π) indices (Table 1), analysis of molecular variance (AMOVA) (Table 2), K2P corrected pairwise molecular divergence matrices and pairwise fixation indices (Table 3) and haplotype networks (Fig. 2). All measures indicate strong genetic partitioning and substantial structure among populations.

Nucleotide diversity indices (π) for both COI and 16S sequences were low. Within islands, values for COI were

Table 1 Genetic variability of COI sequences ($n = 276$, 645 bp) in *Succinea caduca*. Nucleotide sequence statistics, haplotype data and population parameters were computed using DNASP (Rozas *et al*. 2003). Populations are grouped according to island

Island (no. of populations sampled)	Kauai (3)	Oahu (12)	Molokai (3)	Lanai (3)	Maui* (2)	Hawaii† (1)	Total (24)
Sample size	34	136	28	33	19	26	276
Mean no. of samples per population	11.33	11.33	9.33	11	9.5	26	11.58
No. of haplotypes (N_h)	21	98	6	12	4	12	153
Haplotype diversity (h)	0.750 ± 0.036	0.989 ± 0.00001	0.765 ± 0.0023	0.932 ± 0.052	0.614 ± 0.028	0.812 ± 0.048	0.810 ± 0.009
Nucleotide diversity (π)	0.0148 ± 0.004	0.0246 ± 0.003	0.0106 ± 0.003	0.0099 ± 0.010	0.0047 ± 0.004	0.0057 ± 0.008	0.0117 ± 0.001
No. of segregating sites (S)	30	118	20	25	4	27	138
F_{ST} among populations	0.242	0.556	0.805	0.592	*	†	0.618
D_{xy} among populations	0.0218	0.0432	0.0177	0.0142	*	†	0.028

*a single snail was sampled at Waikapu (V), Maui.
†a single population, Makeahua Gulch (X) was sampled on Hawaii.

Table 2 Analysis of molecular variance (AMOVA) results produced using ARLEQUIN (Excoffier *et al*. 2005)

Source of variation	Sum of squares	Variance components	Percentage of variation	Fixation indices
Among islands	309.050	1.00839 Va	11.50	$\Phi_{CT} = 0.115$
Among populations within islands	666.909	4.21726 Vb	48.11	$\Phi_{SC} = 0.544$
Within populations	644.316	3.54020 Vc	40.39	$\Phi_{ST} = 0.600$
Total	1620.275	8.76585	100.0	

Significance tests based on 1023 permutations:
Va and Φ_{CT}: P(rand. value ≥ obs. value) = 0.00684 ± 0.00231.
Vb and Φ_{SC}: P(rand. value ≥ obs. value) < 0.00001 ± 0.00001.
Vc and Φ_{ST}: P(rand. value ≤ obs. value) < 0.00001 ± 0.00001.

0.0047–0.0246 (mean 0.0117, SD 0.0009) and for 16S were 0.003–0.020 (mean 0.0276, SD 0.0011). There were 153 unique COI and 53 unique 16S haplotypes. Shared haplotypes among populations (Fig. 2) were rare and were only observed for COI, and only in three instances: one haplotype was shared between two snails from Russian Fort (B) and Port Allen (C), Kauai; one was shared among 10 snails from Barking Sands (A) and eight snails from Russian Fort (B), Kauai; and one was shared between a snail from Koko Crater (N) and two from Kailua (I), Oahu. No COI haplotypes were shared among islands. No 16S haplotypes were shared among populations. Overall haplotype diversity (h) for COI ($n = 24$ populations) was 0.614–0.989 (mean 0.810, SD 0.002) and for 16S ($n = 16$ populations) was 0.90–1.0 (mean 0.994, SD 0.003). Thus, an overall pattern of high haplotype diversity (h) and low nucleotide diversity (π) was observed for both gene fragments.

Corrected (K2P) pairwise molecular divergence values within populations were 0–0.027 (mean 0.009), whereas divergences between populations were substantially higher (mean 0.028), with a maximum value of 0.058 between Lualualei Valley (G), Oahu (Fig. 1), and Makeahua Gulch (X), Hawaii, a distance of about 300 km. The overall mean for between-population comparisons within islands was 0.026, not significantly different from the overall mean. The maximum value for pairwise individual sequence comparisons was 0.073. Average pairwise molecular

Table 3 Average pairwise F_{ST} (below diagonal), and corrected average genetic distance D_{xy} (above diagonal) based on 276 COI sequences from 24 populations grouped by island. Sequences from Lanai, Molokai and Maui were grouped and are shown as Maui Nui, since for 75% of the past 1.2 million years, they were a single island (see Price & Elliott-Fisk 2004). A close genetic relationship between Oahu and Maui Nui snails is evident, possibly enhanced by gene flow mediated by the former land bridge connection between east Oahu and Maui Nui. Fixation indices were computed using DNASP (Rozas *et al*. 2003); molecular divergence values were generated using the K2P substitution model in MEGA (Kumar *et al*. 2004)

	Oahu	Kauai	Hawaii	Maui Nui
Oahu	—	0.02807	0.04789	0.02108
Kauai	0.31855	—	0.04539	0.02512
Hawaii	0.68387	0.78744	—	0.04539
Maui Nui	0.10557	0.46800	0.79341	—

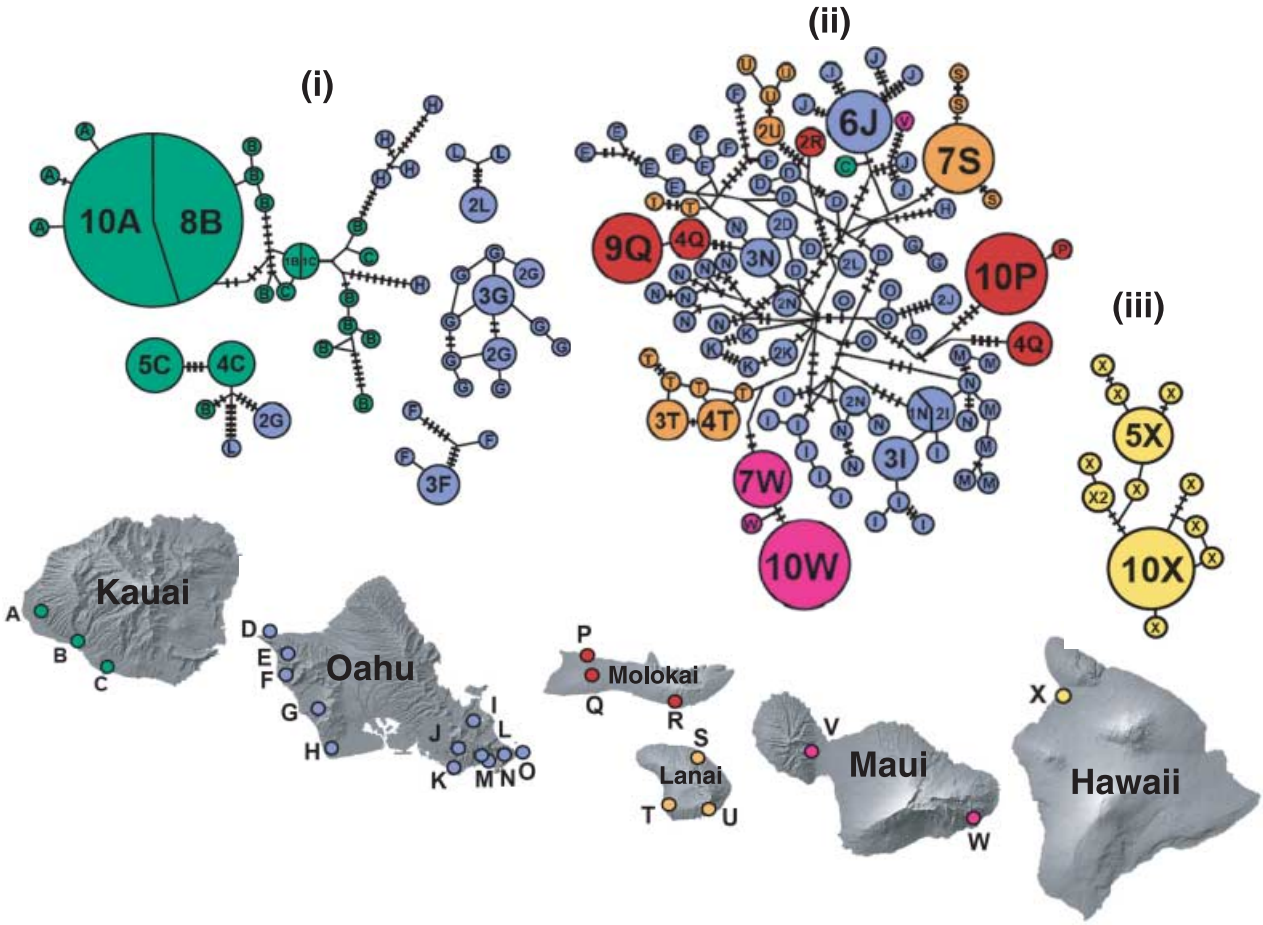

Fig. 2 Haplotype networks for COI from 24 populations produced using TCS version 1.18.mac (Clement *et al*. 2000). Statistical parsimony analysis with a 95% connection limit resulted in seven unconnected minimum spanning networks. Circles represent haplotypes, letter designations represent sampling sites, numbers, where present, show number of snails sharing that haplotype. Circles lacking numbers represent one specimen. Dash marks along branches represent unsampled haplotypes, a branch that separates two circles indicates a difference of one substitution. Network labels (i)–(iii) reflect clades with the same designations in Fig. 4. Islands not shown to scale; see Fig. 1 for distances between islands, relative island sizes, and sample locality details.

divergences between islands were 0.021–0.048, the lowest value being between Maui Nui (Molokai, Lanai, Maui) and Oahu (Table 3).

Overall between-population F_{ST} values for COI sequences were 0.092–0.991 (mean 0.618). Pairwise F_{ST} values for between-island comparisons were 0.106–0.684, the lowest value being between Maui Nui and Oahu (Table 3).

Among-island F_{ST} values based on 84 16S sequences (GenBank Accession nos EF217222–217304) from a subset of 16 of the 24 populations were 0.408–0.845 (mean 0.612).

Values of Nei's coefficient of gene differentiation G_{ST} (Nei 1982) for COI were 0.0014–0.4049 (mean 0.0867). Mean numbers of migrants (Nei 1982) per generation among populations inferred from haplotype partitioning patterns and sequence diversity data were low, 1.00 and 0.11, respectively. Although neutrality test statistics were uniformly negative, not all were statistically significant. Tajima's D was not significant for the COI data set ($D = -1.215$, $P > 0.1$), indicating no evidence of selection on this locus and a neutral model could not be rejected (Tajima 1989). Fu and Li's D^* and F^* (Fu & Li 1993), however, were negative and showed significant departures of haplotype diversity from neutral expectations, and provided evidence of population expansion ($D^* = -3.137$, $P < 0.05$; $F^* = -2.599$, $P < 0.05$). Mismatch distribution plots violated expectations under a constant population size equilibrium distribution model ($R^2 = 0.0566$) but were consistent with a sudden population expansion model (Fig. 6) (see Rogers & Harpending 1992).

Analysis of molecular variance (AMOVA)

AMOVA revealed that 206 of 210 (98%) pairwise population F_{ST} comparisons for COI were statistically significant. Sequence-based F_{ST} values for COI were generally high between populations, the mean being 0.566 (range 0.092–0.991).

While there was a significant effect of island on genetic partitioning (Φ_{CT} = 0.115, P = 0.00684), estimates of inferred gene flow were higher among islands than among populations within the same island. The lowest hierarchical variance component was among islands (Va = 11.5%) (Table 2). Partitioning of molecular variance among populations within islands was highly significant (Φ_{SC} = 0.544, $P < 0.001$) and the majority of hierarchical molecular variance was accounted for by within-island, among-population comparisons (Vb = 48.11%). AMOVA patterns were inconsistent with expectations under an isolation-by-distance model, in which nearby populations would be most closely related, and populations from the same volcano and or island would be more closely related than those from different islands.

Statistical parsimony

Statistical parsimony analysis of the 276 COI fragments produced seven networks representing 153 unique haplotypes, only three of which were shared among populations (Fig. 2). Separate networks differed by at least 32 substitutions. Haplotype networks suggest population diversification patterns that do not correspond directly to geographic location. An abundance of private haplotypes is apparent, particularly for east Oahu (Fig. 2).

Isolation-by-distance analysis

A nonparametric Mantel test provided no evidence for nonrandom associations between genetic and geographic distance matrices (R^2 = 0.0876, $P > 0.05$) (Fig. 3). Rather than adhering to an island model of population genetic variation, genetic distances varied in a manner independent of geographic proximity or island of origin.

Phylogeny reconstruction

For combined COI/16S analysis, a partition homogeneity test implemented in PAUP* (Swofford 2002) with a heuristic search, and 1000 replicates based on 184 parsimony informative characters, resulted in a P value of 0.01, suggesting conflicting topologies. However, trees produced for 16S and COI independently were monophyletic and largely congruent with the main conflict being minor rearrangements within clades. In the interest of maximizing phylogenetic signal and number of phylogenetically informative sites, we analysed the combined data set (see Yoder *et al*. 2001). Using MODELTEST (Posada & Crandall 1998), HKY+I+G (Hasegawa *et al*. 1985) was identified as the most appropriate model, with gamma distributed rates, number of invariant sites, ti/tv ratio, and the shape parameter of the gamma distribution optimized to the data. A heuristic maximum-likelihood (ML) search was conducted using the total 1096 bp of COI and 16S, with molecular clock not enforced, five tree-bisection–reconnection replicates, shape parameter (α) 0.1879, ti/tv 1.26779, 276 distinct data patterns under this model, four rate categories, and a random addition sequence. Empirical base frequencies were A = 0.2958, C = 0.1300, G = 0.1626, T = 0.4116. For bootstrap analyses, maximum parsimony (MP) and minimum evolution (ME) were used for the concatenated COI/16S data set. MODELTEST identified the Kimura 3-parameter model (K3P) (Kimura 1981) as the second best-fit model, with a nearly identical likelihood score to the best-fit model HKY+I+G, so K3P was used with the ME optimality criterion. Resulting ML, ME and MP topologies were congruent, with the main differences being rearrangements among branch tips. Therefore, the topology recovered by a heuristic ML search is shown with ME/MP bootstrap values on nodes common to all three approaches (Fig. 4). For the full COI data set an unrooted MP cladogram was generated with a heuristic search (Fig. 5).

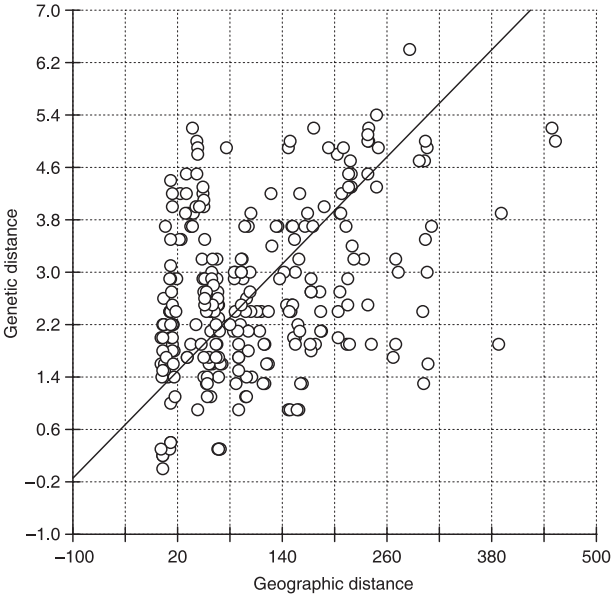

Fig. 3 Graph of the correlation of genetic and geographic distance: Z = 822.3700, R^2 = 0.0876, r = 0.2960, one-sided $P > 0.05$ based on 3000 randomizations (for test of negative correlations, one-sided P = 0.9450) and a reduced major axis regression to calculate intercept and slope of genetic distance vs. geographic distance (user-entered K2P corrected COI distances).

Discussion

Patterns of molecular diversity

Our analyses of geographic patterns of mtDNA variation show that populations of *Succinea caduca* are highly genetically structured. Overall, haplotype diversity is high for both COI and 16S genes (COI: h = 0.810; 16S: h = 0.977). Of 276

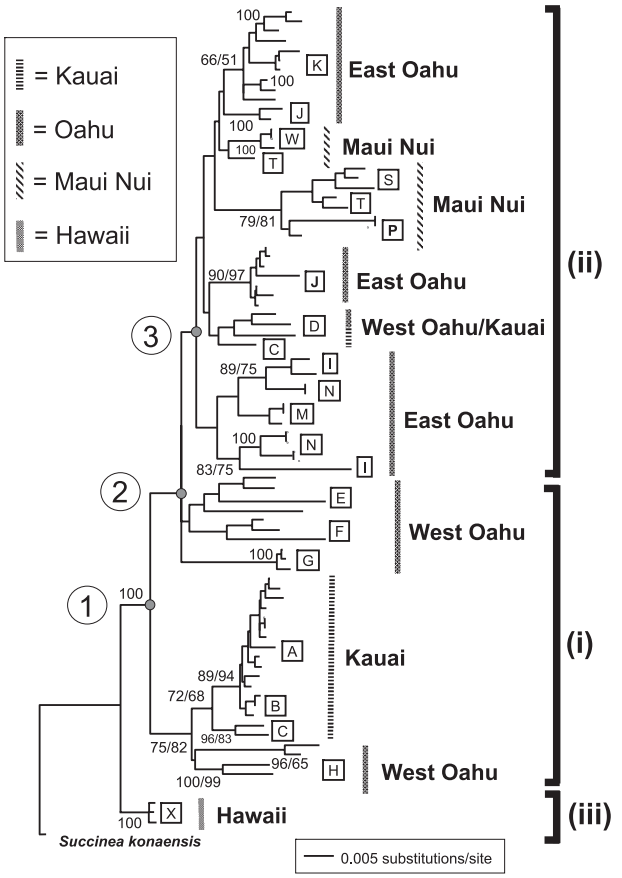

Fig. 4 Area ML phylogram based on a concatenated 1096 bp fragment of the COI and 16S mtDNA genes, from 85 specimens, including a subset of 84 *Succinea caduca* from 16 populations on six islands and a single *Succinea konaensis* to root the tree. Node support values were generated via 1000 bootstrap replicates, shown as ME/MP. Where both approaches produced identical support a single value is shown. Population codes are boxed and shown beside ingroup clades centred over the appropriate node, with islands shown to the right. Patterned bars indicate islands, and major nodes are numbered corresponding to mismatch distribution peaks (Fig. 6), labels (i)–(iii) correspond to haplotype networks (Fig. 2).

COI sequences, just over half the haplotypes are unique and most (98%) are private by population. Only three haplotypes are shared among populations, and only within islands. No 16S haplotypes are shared among populations. However, for both genes nucleotide diversity is low (COI: $\pi = 0.012$; 16S: $\pi = 0.0216$). The overall pattern of high haplotype diversity (h) and low nucleotide diversity (π) imply that independent founder events resulted in multiple unique populations that each persisted in isolation for sufficient time to allow accumulation of substitutions through drift. This pattern is also evident in the cladograms, in which sequences from individual populations usually group together (Figs 2, 4 and 5), yet island populations do not necessarily do so. Dispersal events have apparently been of sufficient frequency to distribute numerous populations among all of the main Hawaiian islands; however, observed levels of geographic partitioning patterns also suggest that each population originated via a unique founder event, and that dispersal frequency is insufficient to counteract the gradual phyletic divergence among isolated populations.

Molecular divergence was found to be low within populations, with a mean K2P distance of 0.009, but significantly higher among populations, with a mean of 0.028. Likewise, F_{ST} values among populations are significant suggesting substantial structure, fixed differences among populations and strong genetic partitioning. Although phylogenetic trees suggest that in many cases populations cluster by island (Figs 4 and 5), AMOVA reveals that the majority of molecular variance is accounted for by within-island population comparisons ($\Phi_{SC} = 0.544$, Vb = 48.11%) rather than among-island comparisons ($\Phi_{CT} = 0.115$, Va = 11.5%). While this result suggests the importance of the variance of within island comparisons, these comparisons are dominated by Oahu populations, since 12 of 24 populations were sampled on Oahu.

Significant negative values of Fu and Li's F^* and D^*, a negative value of Tajima's D and peaked mismatch distributions suggest past population size changes. All but two (populations A, P) of the 23 mismatch distribution plots showed a poor fit with expectations under a constant population size model. The remaining 21 suggest sudden population expansions following bottlenecks, in which the slope of the leading edge of the curve is inversely correlated with the time since expansion and the minimum population size during the bottleneck, and the height of the wave reflects the increase in population size during recovery (Rogers & Harpending 1992). When a single mismatch distribution was produced for all 23 populations together, three steep peaks are evident (Fig. 6), suggesting that there have been three periods dominated by bottlenecks in the evolutionary history of this species. Possible forces leading to population bottlenecks in the Hawaiian Islands include volcanic eruptions and associated lava flows and fires, Pleistocene sea level rise and climate variation, and colonization by new predators or competitors, all of which could reduce and fragment populations (Carson & Templeton 1984; Gillespie & Roderick 2002; Price & Elliott-Fisk 2004). Also, when a species expands into new habitats or new islands, bottlenecks result if the founding population is small (Carson & Templeton 1984). The three peaks in the mismatch distribution (Fig. 6) may represent three periods of high dispersal, with colonization and expansion associated with the founding of new populations.

Evolutionary explanations for observed patterns

Succinea caduca is a small, hermaphroditic, short-lived land snail that lays sticky jelly like egg masses during the wet

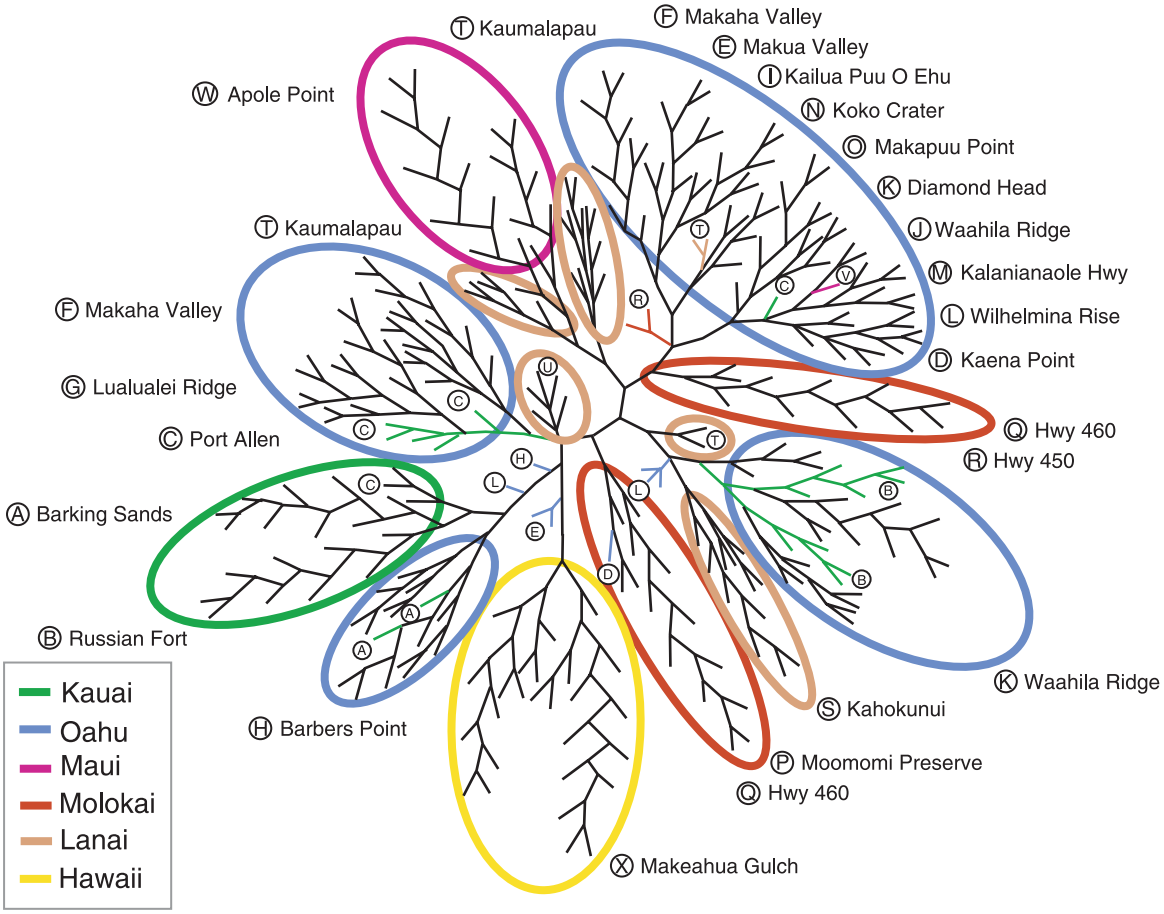

Fig. 5 Unrooted parsimony cladogram for 645 bp fragments of COI with 153 haplotypes from 24 populations of *Succinea caduca*. Island origins are indicated by coloured oval lines. Population names are included with their codes (circled letters) beside clades, with the exception of populations U Manele Bay, Lanai and V Waikapu, Maui, which were not included due to space constraints. For clades consisting of mixed populations, branches are coloured according to island.

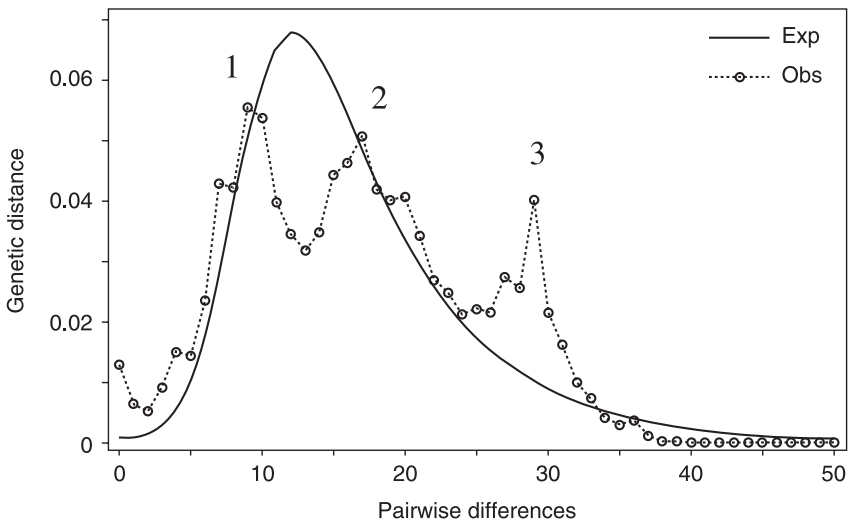

Fig. 6 Frequency distribution of pairwise differences in COI sequences ($n = 276$) showing expectations under a single expansion. The observed values suggest trimodal population expansion, with peaks labelled 1, 2 and 3 (under constant population size expectations $R^2 = 0.0566$, θ initial = 7.102, θ final = 1000).

season. Most of the populations sampled occur in some of the harshest habitats in the Hawaiian Islands. During dry weather conditions, typical in these xeric areas, live *S. caduca* can only be found in deep crevices, under lava boulders or within the bases of thick clumps of dry grass, where temperatures are lower and humidity is elevated and where the snails survive by sealing their shells to flat substrates and remaining inactive. Apparently, they become active for brief periods during and following heavy sustained seasonal rainfall. In areas where live *S. caduca* were found, accumulations of dry, dead shells were also observed, indicating massive seasonal die-offs. In fact, 'caduca' in Latin means 'fallen' or 'perished', perhaps because early naturalists working in Hawaii found accumulations of dead shells.

The population genetic patterns observed suggest episodic dispersal, colonization, founder events and rapid expansions, violating neutral expectations and constant population size predictions. While selective sweeps can produce departures from neutral expectations in terms of mismatch distributions, strong purifying selection results in haplotype homogeneity and lower-than-expected haplotype diversity (Harpending *et al*. 1998); this was not observed in *S. caduca*, in fact the opposite was seen. The observed phylogeographic patterns may have been enhanced further by life-history traits that include cycles of high mortality following sudden expansions, in which diverse, closely related assortments of haplotypes survive through each generation, each spatially restricted population has slightly divergent sets of surviving haplotypes, and drift is the dominant force (see Carson & Templeton 1984). Thus, particularly for populations from marginal leeward habitats such as west Oahu and Kauai, where haplotype analysis resulted in multiple separate clades (Fig. 2) and lower haplotype diversity, these populations may continue to undergo small-scale, possibly seasonal bottlenecks together with a lack of short distance dispersal, resulting in fixation of haplotypes and decreased diversity.

Habitat fragmentation caused by lava flows represents another mechanism leading to population diversification across small spatial scales and enhances haplotype diversity (h) in Hawaiian terrestrial invertebrates (Carson & Templeton 1984; Vandergast *et al*. 2004). The island of Hawaii is the only island with active volcanism, where lava flows are estimated to cover surfaces at rates of 40–90% per 1000 years (Carson *et al*. 1990). Following eruptions, gravity-driven flows blanket and divide mesic coastal areas downslope of the active vent, fragmenting and isolating terrestrial populations of species with limited dispersal ability, such as snails.

The islands of Lanai, Molokai and Maui (Maui Nui) were connected for about 75% of their 1.2-million-year history (Price & Elliott-Fisk 2004), and east Oahu was connected to Molokai via an Early Pleistocene land bridge for several hundred thousand years (Carson & Clague 1995). Consistent with predictions of certain authors (Funk & Wagner 1995; Price & Clague 2002) and phylogeographic results of a few previous studies (Piano *et al*. 1997; Jordan *et al*. 2005) our results (Fig. 2, Table 3) demonstrate the importance of Pleistocene land bridges and historical island-habitat availability in maintaining interisland gene flow among east Oahu and the islands of Maui Nui. The now-submerged areas would have been low-altitude habitat probably suitable for *S. caduca*. In the case of *S. caduca*, we see some partitioning between east and west Oahu but a strong connection between east Oahu and Maui Nui, with all east Oahu haplotypes (except four from population L) belonging to the single network containing all the Maui Nui haplotypes (Fig. 2, network (ii), Fig. 4, clade (ii)). It has been predicted that Pleistocene sea-level changes and restricted habitat availability of Maui Nui should produce low genetic diversity within that group of islands (Jordan *et al*. 2005). This pattern is borne out in Fig. 2 and Table 3.

Interpretation of mismatch distribution plots in relation to underlying phylogenetic patterns and pairwise molecular divergences provides additional insights into the regionally broad distribution and evolutionary history of *S. caduca*. The three peaks correspond to approximate maximum pairwise divergences of 0.055, 0.051 and 0.04 (Fig. 6, peaks 1, 2, 3), tentatively suggesting correlation with the three labelled nodes in the combined COI and 16S phylogram (Fig. 5). Thus, multiple lines of evidence suggest three major cladogenic events, perhaps corresponding to historical periods characterized by favourable conditions for dispersal and range expansion, or representing periods of active volcanism. Genetic diversity in this species may have been maintained by differential survival and volcanic activity throughout its range.

Passive dispersal

Our results challenge the long-standing biogeographic notions that land snails are poor dispersers and that vicariance is the most important force determining the distributions of terrestrial species, as discussed by Cowie & Holland (2006). Haplotype networks (Fig. 2) and area cladograms (Figs 4 and 5) suggest that passive dispersal/colonization among islands occurred multiple times, although, as suggested above, Pleistocene land-bridge connections were important in shaping populations on islands that were once connected.

Isolation-by-distance analysis showed that the geographic pattern of genetic population structure does not conform to a stepwise or island-hopping model of haplotype distributions, and therefore did not result solely from continuous active dispersal by crossing land bridges and/or other historical island connections. It revealed instead

a pattern influenced by punctuated passive dispersal, perhaps mediated by vectors such as hurricanes and birds. This is the first demonstration of episodic dispersal among the Hawaiian Islands in a land snail.

Birds can be important in the transport of snails (Gittenberger *et al*. 2006), including succineids (Anonymous 1936; Rees 1965; Boag 1986). Although passive dispersal of *S. caduca* has not been observed, movement of eggs and/or snails attached to birds may have been important in its dispersal between ridges, valleys and islands.

Snails may also be blown in the wind, either alone or attached to leaves or other parts of plants. Kirchner *et al*. (1997) suggested that small snails such as *Truncatellina* sp., starting from 100 m altitude, could travel several kilometres in strong winds. Assuming that strike frequencies of hurricane and tropical storm-strength winds in the Hawaiian Islands have remained relatively constant, as they have over the past century (Chu & Wang 1998), among-island passive dispersal by wind may have been an important mechanism leading to the distribution of small snails such as *S. caduca* (see Cowie & Holland 2006).

In simple laboratory trials (B.S.H., unpublished), live *S. caduca* attached to tree bark were placed in a salt water aquarium to test their tolerance in water with a salinity of 35 ppt (parts per thousand). After 12 h of immersion, all specimens were alive, indicating that sea water is not immediately lethal and suggesting the potential for rafting between islands on logs and vegetation. Many of the populations sampled were within tens of metres of beachheads, often in xeric areas in or near riparian habitat, suggesting a possible role of periodic flow of runoff down gulches and river beds into the ocean as a dispersal mechanism.

Phylogeography

The Hawaiian Islands were formed as the Pacific plate moves northwestwards over a stationary hot spot in the underlying mantle, which from time to time sends magma up through the plate, resulting in a chain of volcanoes, each volcano sequentially younger than the one that preceded it, and which has now moved northwestwards away from the hot spot (Carson & Clague 1995). Thus, Kauai is the oldest of the main islands (5.1 million years) and the island of Hawaii is the youngest (0.43 million years) (Fig. 1). Kauai and Hawaii have never shared an above-water connection with any other island known to harbour *S. caduca*. Connections between Oahu and Maui Nui permitted flightless and poorly dispersing species to move easily from older to younger volcanoes as the latter were formed (Price & Elliott-Fisk 2004).

The present distribution of *S. caduca* on the islands of Maui Nui and Oahu could therefore be explained by active dispersal among connected islands and vicariant diversification of populations as the islands separated as a result of erosion and subsidence. Some evidence for this pattern is seen in the phylogenetic trees and parsimony networks (Figs 2, 4 and 5). Populations on Kauai and Hawaii had to result from over-water dispersal. For example, a close relationship among sequences from populations at Barbers Point (Oahu, H) and Russian Fort and Port Allen (Kauai, B and C), two islands that have never been connected, suggests over-water dispersal.

Phylogenetic reconstructions for *S. caduca* show a cohesive evolutionary lineage in a patchy geographic mosaic on six islands. In a growing global succineid molecular data set (Holland & Cowie unpublished), *S. caduca* is not the oldest Hawaiian species. It groups with two morphologically similar upland taxa from the island of Hawaii, *Succinea konaensis* Sykes, 1897 and *Succinea quadrata* Ancey, 1904, and represents the most derived subclade within the main succineid clade, 'Clade B' of Rundell *et al*. (2004), which contains only species from the island of Hawaii. Our molecular evidence provides no indication that *S. caduca* is older than these species from the island of Hawaii, resulting in a historical biogeographic pattern that is challenging to explain. The assumption that the basal node within the *S. caduca* lineage arose on the island of Hawaii places a maximum age on the lineage of less than 0.43 million years (the maximum age of the island). Therefore, the corrected (K2P) maximum pairwise genetic divergence within this lineage, 7.3%, arose within this time, necessitating a COI substitution rate of 17% per million years. The basal position of population X within *S. caduca* may conceivably be a result of hybridization with another unsampled island of Hawaii species, but this seems unlikely since in all our analyses of Hawaiian succineids, population X remains within the *S. caduca* ingroup and does not group with any other sampled species. A more likely scenario is that *S. caduca* arose on an older island, a pattern seen in other radiations (e.g. Roderick & Gillespie 1998; Holland & Hadfield 2004), but that populations with older basal haplotypes have either gone extinct or were not sampled. According to this hypothesis, if Kauai were the geographic source, the maximum lineage age is 5.1 million years and the COI substitution rate would be 1.4% per million years, a more reasonable rate estimate.

Although the historical distribution of *S. caduca* is unknown, its coastal, lowland, dry habitat preference and dispersal ability might explain its long-term persistence and wide distribution, as this ancient habitat type has accounted for the majority of island area regardless of an island's stage of geological development.

Conservation and management implications

It has been estimated that Hawaiian dry forests have been reduced by 90% (Noss & Peters 1995). Clearing and burning of lowland dry forests began with the arrival of

the Polynesians; the last remnants are threatened today by residential and golf course development, highway construction and burning. Although most larger fragments of relatively intact dry forests are in montane areas, *S. caduca* is found in coastal regions, usually below 100 m above sea level but occasionally up to about 350 m altitude. Thus, the native habitat of *S. caduca* is under severe threat and the species is not protected legally.

Unlike some of the better known endemic Hawaiian land snails, such as members of the tree snail genus *Achatinella*, which is listed as endangered by the US government (USFWS 1981), and which require native plants on which to live, *S. caduca* is found in both pristine dry forest and disturbed areas, including urban settings, often associated with invasive shrubs and trees. On one hand, this is positive in terms of its survival prospects and ability to persist in the face of anthropogenic habitat alteration. On the other hand, its small size and cryptic nature coupled with its episodic, ephemeral life history, render it vulnerable since it is unlikely to be detected during environmental impact surveys.

One of the challenges of managing *S. caduca* is its distribution in urban areas. For example, we discovered populations of *S. caduca* living in vacant lots within the city limits of Honolulu. In one such instance, there were several other native snails (e.g. *Tornatellides* spp.) coexisting beside invasives such as *Bradybaena similaris* (Rang, 1831), *Achatina fulica* Bowdich, 1822, *Gastrocopta servilis* (Gould, 1843) and *Paropeas achatinaceum* (Pfeiffer, 1846). The presence of endemic species on private property within the city limits is a potentially a positive sign, but also presents unique challenges in terms of jurisdiction.

Population structure data can be useful in management decision-making for Hawaiian land snails (Holland & Hadfield 2002). Genetic data such as those presented here have conservation relevance and are helpful in defining evolutionarily significant units (ESUs), particularly if fine-scale population structure is detected in spatially restricted species. Management efforts focused on genetically defined ESUs can preserve not only biological diversity, but also ongoing natural evolutionary processes. Given the evidence for isolation of divergent *S. caduca* populations, such populations are good candidates for incipient speciation (see Carson & Templeton 1984). Data gathered in this study do not support management of *S. caduca* by island, as inferred population diversification patterns do not always suggest contemporary gene flow within islands. For instance, although all seven geographically proximate populations from east Oahu (I, J, K, L, M, N, O) appear in an unbroken haplotype network, three populations from west Oahu (D, E, F) and all Maui Nui populations were also included in this clade (Fig. 2). Two populations from west Oahu (G, H) grouped with two networks of two and three populations from Kauai (G with B, C; H with A, B, C). On the other hand, 18 snails from two adjacent populations from Kauai (A, B) shared a single haplotype, indicating recent connectivity. Finally, certain populations such as L (east Oahu) consisted of haplotypes with multiple geographic affinities, in this case five haplotypes distributed among three unconnected networks. Genetic diversity patterns and isolation-by-distance analysis show that attempts to manage *S. caduca* populations according to geographic proximity will not maximize genetic diversity, since regional groups of adjacent populations do not necessarily share a most recent common ancestor. Instead effective management decisions for this species will require careful consideration of complex patterns of geographic population diversification such as those revealed in this study.

Acknowledgements

For field assistance, we thank Ken Hayes, Chris Bird, Hiromi Nagatsuka, Vince Costello, Ginny Cowie, Marty Meyer, Stephanie Joe, Nick Kalodimos, Mashuri Waite, Nick Velasco and Hank Oppenheimer. For laboratory assistance we thank Chuong Tran and Masaya Tanaka. We thank The Nature Conservancy, Molokai, for permission to sample in the Moomomi Preserve and for use of their off-road vehicle. An early draft of this paper was improved by the comments of four anonymous reviewers. This project was supported by US National Science Foundation grant DEB-0316308. This paper is dedicated to the enduring inspiration, memory, and legacy of Hampton L. Carson (1914–2004).

References

Anonymous (1936) *Succinea* carried by a bird. *The Nautilus*, **50**, 31.

Boag DA (1986) Dispersal in pond snails: potential role of waterfowl. *Canadian Journal of Zoology*, **64**, 904–909.

Carson HL (1987) Colonization and speciation. In: *Colonization, Succession and Stability* (eds Gray AJ, Crawley MJ, Edwards PJ), pp. 187–205. Blackwell, Oxford.

Carson HL, Clague DA (1995) Geology and biogeography of the Hawaiian Islands. In: *Hawaiian Biogeography: Evolution on a Hot Spot Archipelago* (eds Wagner WL, Funk VA), pp. 14–29. Smithsonian Institution, Washington, D.C.

Carson HL, Kaneshiro KY (1976) *Drosophila* of Hawaii: systematics and ecological genetics. *Annual Review of Ecology and Systematics*, **7**, 311–345.

Carson HL, Lockwood JP, Craddock EM (1990) Extinction and recolonization of local populations on a growing shield volcano. *Proceedings of the National Academy of Sciences, USA*, **87**, 7055–7057.

Carson HL, Templeton AR (1984) Genetic revolutions in relation to speciation phenomena: the founding of new populations. *Annual Reviews of Ecology and Systematics*, **15**, 97–131.

Chu PS, Wang J (1998) Modeling return periods of tropical cyclone intensities in the vicinity of Hawaii. *Journal of Applied Meteorology*, **37**, 951–960.

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1660.

Cowie RH (1996) Variation in species diversity and shell shape in Hawaiian land snails: *in situ* speciation and ecological relationships. *Evolution*, **49**, 1191–1202.

Cowie RH (2001) Invertebrate invasions on Pacific islands and the replacement of unique native faunas: a synthesis of the land and freshwater snails. *Biological Invasions*, **3**, 119–136.

Cowie RH, Holland BS (2006) Dispersal is fundamental to evolution on oceanic islands. *Journal of Biogeography*, **33**, 193–200.

DeSalle R (1995) Molecular approaches to biogeographic analysis of Hawaiian Drosophilidae. In: *Hawaiian Biogeography: Evolution on a Hot Spot Archipelago* (eds Wagner WL, Funk VA), pp. 72–89. Smithsonian Institution, Washington, D.C.

Eldredge LG, Evenhuis NL (2003) Hawaii's biodiversity: a detailed assessment of the numbers of species in the Hawaiian Islands. *Bishop Museum Occasional Papers*, **76**, 1–28.

Excoffier L, Laval G, Schneider S (2005) ARLEQUIN version 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47–50.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**, 294–299.

Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, **147**, 915–925.

Fu YX, Li WH (1993) Statistical tests of neutrality of mutations. *Genetics*, **133**, 693–709.

Funk VA, Wagner WL (1995) Biogeographic patterns in the Hawaiian Islands. In: *Hawaiian Biogeography: Evolution on a Hot Spot Archipelago* (eds Wagner WL, Funk VA), pp. 379–419. Smithsonian Institution, Washington, D.C.

Garey JR, Schmidt-Rhaesa A, Near TJ, Nadler SA (1998) The evolutionary relationships of rotifers and acanthocephalans. *Hydrobiologia*, **387**, 83–91.

Gillespie RG, Roderick GK (2002) Arthropods on islands: colonization, speciation and conservation. *Annual Reviews of Entomology*, **47**, 595–632.

Gittenberger E, Groenberg DSJ, Kokshoom B, Preece RC (2006) Molecular trails from hitch-hiking snails. *Nature*, **439**, 409.

Goodacre S (2002) Population structure, history and gene flow in a group of closely related land snails: genetic variation in *Partula* from the Society Islands of the Pacific. *Molecular Ecology*, **11**, 55–68.

Gulick JT (1905) Evolution, racial and habitudinal. *Carnegie Institution of Washington Publication*, **25**, 1–269, pls. 1–5.

Hadfield MG, Miller SE, Carwile AH (1993) The decimation of endemic Hawai'ian [sic] tree snails by alien predators. *American Zoologist*, **33**, 610–622.

Harpending HC, Batzer MA, Gurven M, Jorde LB, Rogers AR, Sherry ST (1998) Genetic traces of ancient demography. *Proceedings of the National Academy of Sciences, USA*, **93**, 1961–1967.

Hasegawa M, Kishino H, Yano Y (1985) Dating the human ape split by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, **22**, 160–174.

Holland BS, Cowie RH (2006) New island records of an endemic Hawaiian land snail species, *Succinea caduca* Mighels, 1845 (Gastropoda: Pulmonata: Succineidae). *Bishop Museum Occasional Papers*, **88**, 58–60.

Holland BS, Hadfield MG (2002) Islands within an island: phylogeography and conservation genetics of the endangered Hawaiian tree snail *Achatinella mustelina*. *Molecular Ecology*, **11**, 365–376.

Holland BS, Hadfield MG (2004) Origin and diversification of the endemic Hawaiian tree snails (Achatinellinae: Achatinellidae) based on molecular evidence. *Molecular Phylogenetics and Evolution*, **32**, 588–600.

Hormiga G, Arnedo M, Gillespie RG (2003) Speciation on a conveyor belt: sequential colonization of the Hawaiian Islands by *Orsonwelles* Spiders (Araneae, Linyphiidae). *Systematic Biology*, **52**, 70–88.

Hudson RR, Slatkin M, Maddison WP (1992) Estimation of levels of gene flow from DNA sequence data. *Genetics*, **132**, 583–589.

Jensen JL, Bohonak AJ, Kelley ST (2005) Isolation by distance, web service. *BMC Genetics*, **6**, 13. http://phage.sdsu.edu/~jensen/.

Jordan S, Simon C, Foote D, Englund RA (2005) Phylogeographic patterns of Hawaiian damselflies (Odonata: Coenagrionidae) correlate with Pleistocene island boundaries. *Molecular Ecology*, **14**, 3457–3470.

Kimura M (1981) Estimation of evolutionary distances between homologous nucleotide sequences. *Proceedings of the National Academy of Sciences, USA*, **78**, 454–458.

Kirchner C, Krätzner R, Welter-Schultes FW (1997) Flying snail — how far can *Truncatellina* (Pulmonata: Vertiginidae) be blown over sea? *Journal of Molluscan Studies*, **63**, 479–487.

Kumar S, Tamura K, Nei M (2004) MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics*, **5**, 150–163.

Murray J, Stine OC, Johnson MS (1991) The evolution of mitochondrial DNA in *Partula*. *Heredity*, **66**, 93–104.

Nei M (1982) Evolution of human races at the gene level. In: *Human Genetics, Part A: The Unfolding Genome* (eds Bonne-Tamir B, Cohen T, Goodman RM), pp. 167–181. AR Liss, New York.

Noss RF, Peters RL (1995) *Endangered Ecosystems: A Status Report on America's Vanishing Habitat and Wildlife*. Defenders of Wildlife, Washington, D.C.

Piano F, Craddock EM, Kambysellis MP (1997) Phylogeny of the island populations of the Hawaiian *Drosophila grimshawi* complex: evidence from combined data. *Molecular Phylogenetics and Evolution*, **7**, 173–184.

Pilsbry HA (1938–1948) *Land Mollusca of North America (North of Mexico)*. Academy of Natural Sciences, Philadelphia.

Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. *Environmental Economics*, **52**, 273–288.

Pimm SL, Moulton MP, Justice LJ (1994) Bird extinctions in the central Pacific. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **344**, 27–33.

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics*, **14**, 817–818.

Price JP, Clague DA (2002) How old is the Hawaiian biota? Geology and phylogeny suggest recent divergence. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **269**, 2429–2435.

Price JP, Elliott-Fisk D (2004) Topographic history of the Maui Nui complex, Hawaii, and its implications for biogeography. *Pacific Science*, **58**, 27–45.

Rees WJ (1965) The aerial dispersal of Mollusca. *Proceedings of the Malacological Society of London*, **36**, 269–282.

Roderick GK, Gillespie RG (1998) Speciation and phylogeography of Hawaiian terrestrial arthropods. *Molecular Ecology*, **7**, 519–531.

Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution*, **9**, 552–569.

Rozas J, Sánchez-Del Barrio JC, Messeguer X, Rozas R (2003) DNASP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, **19**, 2496–2497.

Rubinoff D, Holland BS (2005) Between the two extremes: mitochondrial DNA is neither the panacea nor the nemesis of phylogenetic and taxonomic inference. *Systematic Biology*, **54**, 952–961.

Rundell RJ, Cowie RH (2003) Growth and reproduction in Hawaiian succineid land snails. *Journal of Molluscan Studies*, **69**, 288–289.

Rundell RJ, Holland BS, Cowie RH (2004) Molecular phylogeny and biogeography of endemic Hawaiian succineid land snails (Pulmonata: Gastropoda). *Molecular Phylogenetics and Evolution*, **31**, 246–255.

Shaw KL (2002) Conflict between nuclear and mitochondrial DNA phylogenies of a recent species radiation: what mtDNA reveals and conceals about modes of speciation in Hawaiian crickets. *Proceedings of the National Academy of Sciences, USA*, **99**, 16122–16127.

Simon C (1987) Hawaiian evolutionary biology: an introduction. *Trends in Ecology & Evolution*, **7**, 175–178.

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. *Genetics*, **139**, 457–462.

Slatkin M, Barton N (1989) A comparison of three indirect methods for estimating average levels of gene flow. *Evolution*, **43**, 1349–1368.

Slatkin M, Hudson RR (1991) Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. *Genetics*, **129**, 555–562.

Solem A (1990) How many Hawaiian land snail species are left? and what we can do for them. *Bishop Museum Occasional Papers*, **30**, 27–40.

Swofford DL (2002) PAUP*: *Phylogenetic Analysis Using Parsimony (*and Other Methods)*, Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, **123**, 585–595.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL X Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, **24**, 4876–4882.

USFWS (1981) Endangered and threatened wildlife and plants; listing the Hawaiian (Oahu) tree snails of the genus *Achatinella* as endangered species. *US Department of the Interior, US Fish and Wildlife Service Federal Register*, **46**, 3178–3182.

USFWS (1993) Recovery plan. O'ahu tree snails of the genus *Achatinella*. *Published by the US Department of the Interior, US Fish and Wildlife Service*. Portland Oregon.

Vandergast AG, Gillespie RG, Roderick GK (2004) Influence of volcanic activity on the population structure of Hawaiian *Tetragnatha* spiders: fragmentation, rapid population growth and the potential for accelerated evolution. *Molecular Ecology*, **13**, 1729–1743.

Vitousek PM (1988) *Diversity and Biological Invasions of Oceanic Islands*. National Academy Press, Washington, D.C.

Wagner WL, Funk VA eds. (1995) *Hawaiian Biogeography. Evolution on a Hot Spot Archipelago*. Smithsonian Institution, Washington D.C.

Wagner WL, Herbst DR, Sohmer SH (1999) *Manual of the Flowering Plants of Hawai'i*. University of Hawaii Press, Bishop Museum Press, Honolulu.

Yoder AD, Irwin JA, Payseur BA (2001) Failure of the ILD to determine data combinability for slow loris phylogeny. *Systematic Biology*, **50**, 408–424

Brenden S. Holland is an Assistant Researcher in the Pacific Biosciences Research Center at the University of Hawaii. His research interests include use of molecular approaches to address phylogeography and conservation of endemic Pacific island radiations, both marine and terrestrial. Robert H. Cowie is a Researcher in the Pacific Biosciences Research Center at the University of Hawaii. His research addresses issues related to the origins and determinants of biodiversity and its conservation, as well as the origins and impacts of introduced species. His primary focus is on non-marine snails in the islands of the Pacific and freshwater snails in South and Central America.
