Islands within an island: phylogeography and conservation genetics of the endangered Hawaiian tree snail
Achatinella mustelina

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Abstract

Mitochondrial DNA (mtDNA) sequences were used to evaluate phylogeographic structure within and among populations of three endangered Hawaiian tree snail species (n = 86). The primary focus of this investigation was on setting conservation priorities for Achatinella mustelina. Limited data sets for two additional endangered Hawaiian tree snails, A. livida and A. sowerbyana, were also developed for comparative purposes. Pairwise genetic distance matrices and phylogenetic trees were generated, and an analysis of molecular variance was performed on 675-base pair cytochrome oxidase I gene sequences from multiple populations of Hawaiian tree snails. Sequence data were analysed under distance-based maximum-likelihood, and maximum-parsimony optimality criteria. Within the focal species, A. mustelina, numbers of variable and parsimony informative sites were 90 and 69, respectively. Pairwise intraspecific mtDNA sequence divergence ranged from 0 to 5.3% in A. mustelina, from 0 to 1.0% in A. livida and from 0 to 1.9% in A. sowerbyana. For A. mustelina, population genetic structure and mountain topography were strongly correlated. Maximum genetic distances were observed across deep, largely deforested valleys, and steep mountain peaks, independent of geographical distance. However, in certain areas where forest cover is presently fragmented, little mtDNA sequence divergence exists despite large geographical scales (8 km). Genetic data were used to define evolutionarily significant units for conservation purposes including decisions regarding placement of predator exclusion fences, captive propagation, re-introduction and translocation.

Keywords: Achatinella, conservation genetics, ESU, Hawaiian tree snails, molecular divergence, phylogeographic structure

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Introduction

The Hawaiian Islands contain the most isolated terrestrial ecosystems on Earth. The nearest landmasses to Hawaii are North America, > 4300 km away, and Japan, > 6400 km away (Coles et al. 1999). The combined evolutionary effects of geographical isolation and habitat diversity have resulted in unparalleled levels of endemism of Hawaiian biota. It is estimated that 95% of the native terrestrial Hawaiian flora and fauna are endemic (Carlquist 1970). Among native Hawaiian land snails, more than 750 valid species are recognized, 99% of which are endemic (Cowie et al. 1995).

The endemic Hawaiian land snail fauna is considered by some researchers to be the most remarkable in the world (e.g. Zimmerman 1948). Among the most distinctive and diverse elements of the Hawaiian land snail fauna are the species within the endemic subfamily Achatinellinae (Pulmonata; family Achatinellidae). The shells of these tree-dwelling snails exhibit a diverse array of colours and banding patterns that have fascinated and confounded scientists and shell collectors for over a century (Gulick 1873; Zimmerman 1948; Cooke & Kondo 1960). Hawaiian tree snails exist in relatively small, fragmented populations and have limited vagility. They are therefore particularly attractive for studies of population structure and speciation, and played a significant role in the early development of evolutionary thought (Gulick...
The endemic Hawaiian tree snails are of great conservation concern due to recent catastrophic rates of extinction. The critically endangered O‘ahu genus *Achatinella* historically comprised one of the most species-rich groups of the endemic Hawaiian gastropod biota (Zimmerman 1948). In 1981, all remaining species of the original 41 valid species, were placed on the US List of Endangered Species under the Endangered Species Act (USFWS 1981). CITES Appendix I. Recent surveys indicate that only eight to 10 (depending on resolution of two systematic discrepancies) species currently remain on the island (M.G.H. unpublished data). For extant species, range reductions have been in excess of 95% in the Ko‘olau Mountains and 75% in the Wai‘anae Mountains (USFWS 1993).

The main threats to tree snails today are from invasive taxa, specifically habitat loss due to ungulates (Pilsbury & Wai‘anae Mountains (USFWS 1993).

Extinct

in this study, the Ko‘olau Range. The two Ko‘olau Range species examined in this study, *A. mustelina* is the only one remaining in the Wai‘anae Mountains, while the other surviving species are found in the Ko‘olau Range. The two Ko‘olau Range species examined in this study, *A. messa* and *A. ouzeriana*, are considered sister taxa, and have limited, patchy, distributions along the summit of the northern portion of the range (USFWS 1993).

A. mustelina, however, has a relatively broad distribution from the southern to northern extremes of the Wai‘anae Range above 600 m elevation. Based on currently available information, *A. mustelina* is the most abundant of the Hawaiian tree snails (USFWS 1993), although its numbers have been severely impacted by invasive species and human activity (e.g. Hadfield & Mountain 1980).

Biodiversity can be defined in terms of genetic diversity (e.g. Avise 1996; Crozier 1997). Consequently, application of phylogenetic methods can be vital to conservation biology by providing information regarding the allocation of genetic variation among populations or taxa (Harvey & Steers 1999). Particularly in situations where a choice among populations for conservation efforts is necessary, information regarding the partitioning of genetic variation may be the single most important criterion to guide that choice (Baverstock & Moritz 1996; Pope 1996). Therefore, knowledge of the phylogeographic structure of fragmented tree snail populations is vital to the formulation of long-term strategies for the conservation and management of remnant species.

In 1998 the US Fish and Wildlife Service (USFWS) issued a formal Biological Opinion recommending stabilization actions in the Wai‘anae Mountains due to various threats to regional communities of 28 endangered species of plants, birds and invertebrates, including *A. mustelina*. Specific areas were identified by USFWS personnel as critical habitat and were set aside for in situ management. The geographical scope of the management plan for *A. mustelina* included the entire distribution of the species throughout the Wai‘anae Mountains. As conservation management and recovery strategies are developed, one of the long-term goals may include construction of multiple predator exclusion fences throughout the range of the snail. Such structures surround existing stands of native trees that harbour tree snail populations. Fence structures could be modelled after two existing installations built by the State of Hawaii and the US Army in the northern Wai‘anae Range. Decisions regarding placement of new fences might be based in part on preserving maximum genetic diversity and maintaining independent lineages, guided by evolutionarily significant units (ESUs) established in this study.

Our hypothesis is that there is some level of geographical structuring of tree snail populations based on reproductive isolation resulting from population fragmentation and limited dispersal. The primary objective of this study was to use mitochondrial DNA (mtDNA) markers to test this hypothesis and evaluate the partitioning of intraspecific genetic diversity, and use the resulting spatial distribution of genetic variation to define ESUs. Various operational definitions of ESUs exist in the literature. In accordance with the ESU definition of Ryder (1986), each ESU defined in this study is comprised of one or a set of populations with a distinct, long-term evolutionary history. In partial fulfilment of the criteria of Moritz (1994), mtDNA gene tree clades defining ESUs exhibit monophyly. ESUs recognized in this study are separable based on clear phylogeographic subdivisions within the species, and consist of genetically cohesive populations that have been isolated from other populations in terms of contemporary gene flow. Ideally, ESUs should be based on multiple concordant characters (Avise 2000). However, the management units defined in this study are at the very least a useful preliminary conservation framework, and clearly merit further investigation. Genetically based ESUs will help guide fence placement.

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captive propagation (Kobayashi & Hadfield 1996), field translocations and the release of captive-bred individuals. If an *A. mustelina* population became extinct, phylogeographic data could be used to direct a reintroduction programme, intended to restore the original genetic condition (Avise 2000).

**Materials and methods**

**Sampling**

Tissue samples were obtained from 18 *Achatinella mustelina* populations representing a hierarchy from closely spaced to most geographically distant sites. Samples were collected between April 1999 and May 2001. Mitochondrial DNA sequences were obtained for 90 O‘ahu tree snail specimens representing a single family (Achatinellidae), two subfamilies (Achatinellinae, Auriculellinae), two genera (*Achatinella* and *Auriculella*) and six species (Table 1). In-group taxa included 69 *A. mustelina* from 18 populations spanning its range in the Wai‘anae Mountains, nine *A. livida* from four populations and eight *A. sowerbyana* from three populations in the Ko‘olau Mountains. Outgroup taxa included two *A. apexfulva*, one *Auriculella ambusta* and one *Auriculella perversa*. Outgroup specimens were collected in the Ko‘olau Mountains, with the exception of *Auriculella ambusta* collected in the northern Wai‘anae Range.

Various species were evaluated as potential outgroups. The basal position of the outgroup taxa used in this investigation in relation to the ingroup sequences was confirmed by the inclusion of several more distantly related taxa in the analysis, including members of other tropical Pacific land snail families such as Partulidae and Succineidae (Holland & Hadfield, manuscript in preparation).

Field sampling of *Achatinella* spp. was conducted using a noninvasive technique, as described by Thacker & Hadfield (2000), under Endangered Species Permit PRT–826600. Tissue samples were placed in 80% ethanol (80% ethanol : 20% water) in the field and transported to the laboratory for nucleic acid extraction. Samples, which could not be extracted immediately, were stored at –70°C. Live animals that were sampled in the field remained in the field, therefore voucher specimens were not retained. Where possible, close-up photography was used to document each snail sampled.

**DNA extraction, polymerase chain reaction (PCR) amplification and sequencing**

Genomic DNAs were extracted using Dneasy™ nucleic acid extraction kits manufactured by Qiagen® (Qiagen Inc.). DNAs were eluted in de-ionized autoclaved water, and stored at –70°C. PCR was performed using a PTC-100™ thermocycler (MJ Research, Inc.). Universal cytochrome oxidase I (COI) primers (Folmer et al. 1994) were found to amplify the target fragment consistently under the following PCR conditions: 2 min at 92°C, 35 cycles of 94°C for 30 s, 40°C for 45 s and 72°C for 1 min, with a final 72°C extension for 7 min. PCR-amplified DNA fragments were purified with QIAquick® spin columns (Qiagen Inc.), according to the manufacturer’s protocol, then checked via agarose gel electrophoresis. The forward strand was cycle-sequenced using PCR primers and an ABI Prism DYE Terminator Cycle

**Table 1**

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Geographic source</th>
<th>No. of populations sampled</th>
<th>No. of specimens sampled / GenBank accession numbers</th>
<th>Subfamily</th>
<th>Endangered status</th>
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<tr>
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<td>1</td>
<td>Auriculellinae –</td>
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<td></td>
<td>AF400448</td>
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</table>

All specimens are members of the Pacific pulmonate family Achatinellidae. The focus of the investigation was on population structure in *Achatinella mustelina*, with additional species included for comparative purposes.

In the last column, + indicates that the species is listed under the US Endangered Species Act.

Sequencing Reaction Kit in a Perkin-Elmer 9700 thermal cycler, and sequenced using an ABI 377 automated sequencer (PE Biosystems).

Sequence analysis
Distance- and parsimony-based methods were used to generate phylogenetic trees. For distance methods, MEGA (molecular evolutionary genetic analysis, version 2b3; Kumar et al. 2001) was used with a Kimura two-parameter model to produce neighbour-joining trees (Saitou & Nei 1987). Neighbour-joining tree nodes and branch lengths were statistically tested using a bootstrap approach (Felsenstein 1985), and an interior branch test, respectively (Kumar et al. 2001). A maximum-parsimony analysis was conducted using PAUP* 4.0b88 (Swofford 1998), with equal weighting, using the heuristic search option with tree bisection reconnection branch-swapping and 100 random additions. Bootstrap support for each node was assessed based on 1000 bootstrap replicates with tree bisection reconnection and 10 random additions. A maximum-likelihood tree was constructed based on the HKY85 model (Hasegawa et al. 1985) with empirical base frequencies using the heuristic search algorithm.

Population structure analysis
Both within- and among-population variation were assessed using distance analyses of per cent sequence divergence in the program MEGA (molecular evolutionary genetic analysis, version 2b3; Kumar et al. 2001). Kimura two-parameter genetic distances were used to correct for multiple substitutions per site and different substitution rates were used for transitions and transversions. Standard errors of distances were calculated according to (Kumar et al. 2001). Once ESUs were defined, ARLEQUIN 2.0 was used to perform an analysis of molecular variance (AMOVA) to partition the amount of genetic variation in a hierarchical fashion, among ESUs, among populations within ESUs, and within populations (Excoffier et al. 1992). Statistical significance of differentiation at the three levels was quantified and tested using ARLEQUIN 2.0 (Schneider et al. 2000).

Results
Sequence data
Sequences of amplified 675-base pair DNA fragments were unambiguously aligned by eye to the COI gene from the pulmonate gastropod Albinaria cornuta (GenBank accession X83390). GenBank accession numbers for partial COI sequences from 69 Achatinella mustelina, nine A. livida and eight A. sowerbyana sequences are found in Table 1.

For A. mustelina sequences, translation using the Drosophila mitochondrial genetic code (Yamazaki et al. 1997; Wilding et al. 1999; Collin 2000) indicated that of the 225 amino acid residues encoded by the COI gene fragment, 13 positions were variable. Thus, 94.2% of the amino acid positions analysed were conserved. For A. livida, there was a single variable amino acid, found at position 41, as well as for A. sowerbyana, at position 66. For both A. livida and A. sowerbyana, 99.6% of the amino acids encoded by the COI fragment analysed were conserved.

The mean transition/transversion ratio was 2.78, and ranged from 0.6 to 6.0. Empirical nucleotide base frequencies were significantly biased to adenine and thymine; mean values were A = 0.284, C = 0.117, G = 0.168, and T = 0.431. This gene fragment is typically A-T rich in other taxa (e.g. Frati et al. 2000), and A. mustelina sequences were no exception, as adenine and thymine accounted for 71.5% of the fragment. The total number of variable nucleotide sites in A. mustelina was 90. Of the 90 variable positions, 11 (12.2%), were first codon position, eight (8.9%), were second codon position, and 71 (78.9%) were third codon position. Including DNA sequences from both outgroup taxa, there were 170 variable nucleotide sites, and base composition values were identical to those found for A. mustelina.

Phylogenetic inference
Regardless of which optimality criterion was used to infer phylogenetic trees from COI sequences, A. mustelina data showed strong statistical support for substantial intraspecific population level divergence, and gene tree topologies were nearly identical under neighbour joining, Minimum Evolution, UPGMA, maximum likelihood and maximum parsimony approaches.

A neighbour-joining phylogram is shown for A. mustelina, A. livida, A. sowerbyana, and outgroups (Fig. 1). Using the neighbour-joining method, A. mustelina was monophyletic with 94% bootstrap support, and the two sister taxa A. livida and A. sowerbyana together comprised a monophyletic clade with 100% bootstrap support (Fig. 1). The subtree for A. mustelina was characterized by two main clades. Clade I comprised populations from the northwest of the mountain range, populations 1–7, consisting of two subclades labelled A and B. Clade II comprised populations from the

Fig. 1 (opposite) Neighbour-joining phylogram inferred from 90 Hawaiian land snail partial COI sequences using a Kimura two-parameter substitution model. Two main clades representing Achatinella mustelina sampled in the Waianae Mountains are indicated as Clade I and Clade II. In-group specimens are labelled according source population, and sample code number. Six A. mustelina subclades are labelled A–F. For Ko‘olau Range taxa, species abbreviations As and Al are Achatinella sowerbyana and A. livida, respectively. Bootstrap values above 50% are shown, and outgroup taxa are indicated.

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CONSERVATION GENETICS OF ACHATINELLA MUSTELINA

Clade I
Northern Wai‘anae Mountains

Achatinella mustelina

Clade II
South-central Wai‘anae Mountains

Achatinella livida
and A. sowerbyana

Ko‘olau Mountains

Outgroup taxa

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central to the southern portion of the mountain range, populations 8–18, with four subclades labelled C–F (Fig. 1). Bootstrap support for the *A. mustelina* subtree was 87% for Clade I and 72% for Clade II. Two individuals from population 10 (Pop 10), individuals 934 and 935, appear basal to the two main clades comprising the subtree of northern populations (Fig. 1). The three remaining sequences from Pop 10 are in a clade consisting of individuals from Pops 8 and 9, in the subclade labelled C. Two additional sequences did not match their source populations Pop 8–959, and Pop 9–859. The sequence from individual Pop 9–859 most closely matched the haplotype of subclade E in Clade II with 60% bootstrap support (Fig. 1). Specimen Pop 8–959 showed the highest affinity to subclade A, with bootstrap support of 64% (Fig. 1).

Relative to the two Ko‘olau Range taxa *A. livida* and *A. soeverbyana*, *A. mustelina* populations show considerably higher population subdivision (Fig. 1). Trees inferred from *A. mustelina* sequence data indicate more ancient population divergence times. In comparison, the deepest nodes of the tree of the two Ko‘olau Mountain species suggest more recent divergence times both within each species as well as between the two species than was observed for *A. mustelina* populations (Fig. 1).

**Genetic structure of populations/ESU designation**

*Achatinella mustelina* populations were found to be highly structured and multiple haplotypes were identified. The overall pattern of genetic variation was characterized by higher among-population variation than within-population variation, and four clusters containing multiple populations (from three to six populations each) with extremely low interpopulation genetic divergence (Table 2). Two additional populations from the southern portion of the range (Pop 17 and 18) were found to be genetically distinct from one another and all other populations sampled (Figs 1 and 2). Based on the geographical distribution of genetic variation in terms of cladistic (Figs 1 and 2), genetic distance (Table 2), and AMOVA (Schneider et al. 2000) (Table 3) six populations or groups of populations were designated as ESUs, labelled A–F (Fig. 1) in accordance with the definition of Ryder (1986), based on evidence of historical isolation (Moritz 1994; Avise 2000). The mean among-population variation was much higher than the mean variation found among populations within an ESU. Genetic distances, also expressed as molecular divergence values (%), were determined using the bootstrap method with 500 replicates and a random number seed. Values were determined based on partial COI sequences using a Kimura two-parameter substitution model (Kumar et al. 2001). Populations comprising the six-distance-based ESUs are as follows: ESU A = 1–3, ESU B = 4–7, ESU C = 8–10, ESU D = 11–16, ESU E = 17 and ESU F = 18.

Divergence values and SE shown in bold print are within-ESU interpopulation means. Note that populations are arranged in roughly north-south, west-east fashion, from 1 to 18 (Fig. 1).

**Table 2** Pairwise genetic distance matrix showing mean inter- and intrapopulation molecular sequence divergence values and standard errors (SE) for 69 specimens from 18 populations of *Achatinella mustelina* sampled in the Wai‘anae Mountains of O‘ahu

<table>
<thead>
<tr>
<th>Pop</th>
<th>ESU A</th>
<th>ESU B</th>
<th>ESU C</th>
<th>ESU D</th>
<th>ESU E</th>
<th>ESU F</th>
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<td>1</td>
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</table>

Within-population mean distances are underlined, shown along the diagonal. Mean among-population distances are below the diagonal. SE values (above diagonal) were computed using the bootstrap method with 500 replicates and a random number seed. Values were expressed as molecular divergence values (%), were determined using a Kimura two-parameter substitution model. Uncorrected pairwise sequence divergence among individuals.
of *A. mustelina* ranged from 0.00 to 0.053 (5.3%). The maximum genetic distance observed between two individuals (0.053 or 5.3%), was more than 10 times the overall within-population mean genetic distance of 0.0048 (0.48%) for this species. Sixteen of 18 *A. mustelina* populations sampled had average intrapopulation distance values of less than 0.008 (0.8%), 15 populations had average intrapopulation distances of 0.006 (0.6%) or less, and for three populations, all individuals characterized were genetically identical. The mean uncorrected pairwise sequence divergence for all *A. mustelina* specimens was 0.029 (2.9%). Table 2 shows the 18 within-population mean values for *A. mustelina*, ranging from 0.00 to 0.019 (1.9%). Average pairwise genetic distances among populations of *A. mustelina* ranged from 0.00 to 0.044 (4.4%) (Table 2).

Mean within-ESU divergence was calculated for the four ESUs composed of more than one population (ESU A-D) (Table 2). The resulting mean within-ESU genetic distance was 0.0026 (0.26%).

For *A. livida* the range of genetic distance among all individuals was from 0.00 to 0.010 (1.0%); and in *A. souerbyana* was from 0.00 to 0.019 (1.9%). Maximum observed intra-specific pairwise distance for either *A. livida* or *A. souerbyana* alone was equal to the highest pairwise distance value between two individuals from each of the two different species (0.019 or 1.9%). Mean intrapopulation distances...
Total 11.299 100.00

<table>
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<tr>
<th>Source of variation</th>
<th>Variance components</th>
<th>Per cent of total</th>
<th>F&lt;sub&gt;CT&lt;/sub&gt;</th>
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<td></td>
<td></td>
</tr>
<tr>
<td>Among populations within ESUs</td>
<td>0.658</td>
<td>5.82</td>
<td></td>
<td>0.233</td>
<td></td>
</tr>
<tr>
<td>Within populations</td>
<td>2.159</td>
<td>19.10</td>
<td></td>
<td></td>
<td>0.809</td>
</tr>
<tr>
<td>Total</td>
<td>11.299</td>
<td>100.00</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Statistical significance of the diversity estimates was assessed using probabilities derived from 1023 permutations, in all cases P < 0.0001.

The operational taxonomic unit labelled ‘1993 captive’ (Figs 1 and 2) represents a specimen from the captive breeding programme in the laboratory of M. G. Hadfield at the University of Hawaii. This specimen was collected in 1993 and sampled in the laboratory in order to confirm our ability to determine the geographical source of individuals housed in the laboratory which were either collected in the field at some time in the past or derive from specimens collected in the field, and raised in the laboratory. This demonstrates that when and if the time comes when release of captive-bred snails is deemed appropriate, we can use mtDNA haplotype data to guide the process in order to maintain the distinct lineages identified.

**Analysis of molecular variance**

Table 3 shows the results of the AMOVA of partial COI sequences and corresponding F-statistics of genetic differentiation. Three sources of variation, grouped hierarchically, were analysed. The highest contribution of molecular variance came from the among-ESU partition (75.1%). Another relevant result of this analysis is the low relative variance contributed by the among-populations within-ESU level (5.8%). Thus, ESUs designated in this study are supported by the AMOVA.

**Discussion**

**Phylogenetics and conservation**

Appropriate and immediate steps are needed to halt the 30-year decline in achatinelline tree snail numbers. In this investigation, hierarchical analysis using molecular markers has revealed multiple haplotypes representing separate lineages in *Achatinella mustelina*. As such, evolutionarily independent phylogroups warrant protection as distinct management units (Solis & Gitzendanner 1999). Data on genetic diversity may be useful in management decision-making in terms of selection of sites for predator-exclusion fences, directing captive-propagation efforts, and carrying out translocations in the field. Once habitat is stabilized and invasive predator populations have been controlled, release of captive-bred specimens may be appropriate. Genetic data will be critical in guiding the release of captive-bred individuals, ensuring that outbreeding depression is
avoided and natural genetic continuities are maintained in the wild.

ESU designation and conservation relevance of phylogenetic findings

The objective of this study was to analyse the genetic variation of A. mustelina populations in relation to their degree of isolation and fragmented distribution in the Wai‘anae Mountain Range on the island of O‘ahu. Three lines of evidence, phylogenetic inference, a genetic distance matrix, and an analysis of intrapopulation genetic diversity, were found to correspond to a pairwise interpopulation divergence value of less than 1.0% (average intrapopulation genetic diversity was 4.4%, and several between-population divergence values were 0.0 (Table 2). However, interpopulation divergence values of zero were only observed among populations within an ESU. In all cases, the lowest among-population genetic distance values were found within ESUs (Table 2).

Population structure/phylogeography

Achatinella mustelina. Molecular genetic data for A. mustelina reveal numerous instances where low genetic distances, values at or below the mean intrapopulation value of 0.0047 (0.47%), persist over relatively long geographical distances following ridge crests in roughly linear patterns (e.g. Fig. 2, ESU D; Table 2). The pattern of population structure probably results from the fact that populations were historically larger and suitable habitat was once more continuous and extensive. Evidence from a variety of sources indicates that the patchy distribution of native host trees in the Wai‘anae Mountains is a relatively recent phenomenon (Pilsbury & Cooke 1912–14). Most forests below 305 m elevation were cleared for agriculture by early Polynesians and later by European settlers (USFWS 1993). Habitat fragmentation at upper elevations has occurred more recently, largely within the last century, as a result of the destruction of underbrush and desiccation of underlying soils due to introduced ungulates (Pilsbury & Cooke 1912–14). Thus the geographical pattern of molecular divergence in A. mustelina may reflect the fact that in the past, large tracts of continuous native forest covered the upper ridges of the Wai‘anae Mountains. Achatinella mustelina appears to have been distributed in widespread, continuous populations that correspond to ESUs identified in this study and these large populations apparently behaved genetically as panmictic units. When forests were more continuous, natural dispersal may have been common within ESUs via tree to tree migration. Populations may have become isolated subsequently, due to factors impacting forest cover and leading to current fragmentation.

In order to account for the observed phylogeographic pattern we propose an evolutionary scenario characterized by three phases, beginning with an initial panmictic phase, followed by long-term, large-scale habitat fragmentation, and finally recent fine-scale fragmentation resulting in the current patchy distribution of A. mustelina. It is clear that at one time in the early geological history of the island, western O‘ahu consisted of a single massive shield volcano, similar in shape to the roughly half-million-year-old volcanoes Mauna Loa and Mauna Kea on the Island of Hawai‘i. The present day position of the summit of Mt Ka‘ala is
shown in Fig. 2. The current terrestrial topography of western O’ahu is characterized by rugged, complex features resulting from the erosive action of wind and rain over 3.7 million years. It is possible that large populations of tree snails gradually became separated from one another by the formation of valleys and ridges, and isolated by distance into the present pattern of ‘islands’ of genetically cohesive populations.

Achatinella livida and A. souverbyana. In an ongoing phylogenetic analysis using COI (cytochrome c oxidase I) sequences (Holland & Hadfield, manuscript in preparation), as well as according to a 16S rRNA phylogeny (Thacker & Hadfield 2000), A. apexfulva has been shown to be a close relative of A. livida, A. souverbyana and A. mustelina. Thacker & Hadfield (2000) found that despite the traditional classification which placed all three of these Ko’olau Range taxa in separate subgenera, ribosomal mtDNA sequence data consistently grouped the three taxa together.

Specimens of A. livida and A. souverbyana can be separated on the basis of chirality and we have never observed them in mixed populations. However, Thacker & Hadfield (2000) concluded that the high degree of genetic similarity between A. livida and A. souverbyana indicates that the taxa may be variants of the same species or are subject to hybridization. COI data presented in this study support the notion that these two taxa are closely related. A. livida and A. souverbyana yielded relatively depauperate genetic profiles, and maximum interspecific divergence was only about 36% (1.9/5.3) of the highest divergence found within A. mustelina. The lower genetic diversity observed among A. livida and A. souverbyana populations, as well as between the two species, suggests a relatively recent evolutionary separation of the taxa. There is evidence that hybridization may have occurred, again casting doubt on the validity of taxonomic divisions that rely on morphological features with unknown genetic bases. On the other hand, in cases where no discernable phenotypic pattern of variation can be detected, a clear pattern of underlying genetic divergence, or cryptic biodiversity may be revealed. The present study provides an example of unambiguous geographical partitioning of genetic variation despite the absence of a clear pattern in morphological diversity. The molecular data presented strongly indicate that currently isolated populations of A. mustelina are evolving independently of one another, and that we are probably witnessing incipient speciation events among fragmented and highly endangered populations. From a conservation biology perspective, this notion underscores the urgency required in addressing the catastrophic loss of achatinelline species, and justifies efforts to preserve the remaining diversity of Hawaiian tree snails. These findings also suggest that further molecular analyses are needed to elucidate intraspecific variation in other achatinelline taxa and to test traditional taxonomic designations among extant species of Hawaiian tree snails. This study demonstrates the utility of molecular markers in defining ESUs for invertebrate conservation. ESUs may be used to maintain the integrity of distinct regional lineages and to conserve a significant fraction of extant genetic diversity within the species.

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This research was carried out as part of an ongoing investigation of conservation genetics, phylogeography and evolutionary relationships among extant achatinella snails from the Hawaiian Islands. Dr Brenden S. Holland is a postdoctoral fellow focusing on the application of molecular phylogeography to conservation issues in the laboratory of Dr Michael G. Hadfield. Dr Hadfield’s research group focuses on various aspects of ecology and conservation biology of endemic Hawaiian tree snails, through use of molecular markers, long-term field studies, and captive propagation.