Patterns and processes in complex landscapes: testing alternative biogeographical hypotheses through integrated analysis of phylogeography and community ecology in Hawai'i

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Abstract

The Island of Hawai'i is a dynamic assemblage of five volcanoes with wet forest habitat currently existing in four distinct natural regions that vary in area, age and geographical isolation. In this complex landscape, alternative assumptions of the relative importance of specific habitat characteristics on evolutionary and ecological processes predict strikingly different general patterns of local diversity and regional similarity. In this study, we compare alternative a priori hypotheses against observed patterns within two distinct biological systems and scales: community composition of wet forest vascular plant species and mitochondrial and nuclear genes of *Drosophila sproati*, a wet-forest-restricted endemic. All observed patterns display strong and similar regional structuring, with the greatest local diversity found in Kohala and the windward side of Mauna Loa, the least in Ka'ū and Kona, and a distinctive pattern of regional similarity that probably reflects the historical development of this habitat on the island. These observations largely corroborate a biogeographical model that integrates multiple lines of evidence, including climatic reconstruction, over those relying on single measures, such as current habitat configuration or substrate age. This method of testing alternative hypotheses across biological systems and scales is an innovative approach for understanding complex landscapes and should prove valuable in diverse biogeographical systems.

Keywords: biogeography, community ecology, *Drosophila*, Hawai'i, hypothesis testing, phylogeography

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Introduction

Observable patterns in biogeographical systems are the result of ecological and evolutionary processes that are heavily influenced by the spatial and temporal characteristics of the associated habitat. These characteristics are often described through simple proxies, such as current habitat patch size and intervening geographical distance, which are then used to infer relevant processes from observed patterns. However, in complex landscapes, these habitat characteristics may be dynamic over space and time, with the associated processes driven by the interaction of a variety of factors. In this case, simple proxies of habitat characteristics may be misleading. This study applies an innovative method for understanding biogeographically complex landscapes, in which alternative general hypotheses of relevant habitat characteristics are tested by integrating observable patterns across multiple biological systems and scales. The resulting biogeographical model of the landscape provides a corroborated general framework for

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interpreting relevant ecological and evolutionary processes and allows for identification of unique biological systems through deviation from the predicted general pattern.

Certain habitat characteristics have been proposed to drive analogous ecological and evolutionary processes to independently produce shared general patterns at the level of species diversity among communities and genetic diversity among populations (Vellend & Geber 2005; Vellend & Orrock 2009). For example, closely adjacent habitat patches can be expected to allow for frequent exchange of individuals, leading to similar species composition among communities as a result of colonizing migrants (Kadmon & Pulliam 1993; Nekola & White 1999) and similar gene frequencies among populations as a result of gene flow (Nei 1972; Slatkin & Barton 1989). In both cases, a single characteristic allowing for frequent migration produces analogous patterns of regional similarity at multiple biological scales. Likewise, large habitat regions are expected to support spatially expansive communities with low extinction rates and therefore high species diversity (MacArthur & Wilson 1967), as well as large population sizes that are thought to buffer stochastic events and permit the maintenance of more alleles (Lande & Barrowclough 1987). Here, again a single habitat characteristic that limits both ecological and genetic drift can produce a shared pattern of high diversity at multiple biological scales. Therefore, the observation of a single pattern shared between biological communities, which respond to ecological processes, and genes in populations, which respond to evolutionary processes, should allow for a general interpretation of relevant habitat characteristics (Vellend 2003, 2010; Chave 2004; Alonso *et al.* 2006). In complex landscapes, the additional use of a priori hypotheses presents a rigorous framework for explicitly testing alternative potential interactions of spatial and temporal habitat characteristics. In this study, we apply this integrated hypothesis-testing approach to the complex landscape of wet forest habitat on the Island of Hawai'i.

The Hawaiian archipelago is 3700 km from the nearest continent and is the result of continental drift over a stationary hot spot in the earth's crust (Wilson 1963; Clague 1996). This isolation and geologic simplicity has long attracted researchers studying a diversity of biological systems and scales as well as the underlying physical geography, resulting in a broad and detailed understanding of the natural history (see Carlquist 1970; Vitousek 1995, 2002; Wagner and Funk 1995). Many ecological and evolutionary studies within the Hawaiian Islands have been informed by this strong and diverse knowledge base. For example, geological data have been relied on for investigations of ecosystem development on new substrate (Crews *et al.* 1995; Vitousek *et al.* 2009) and for relating phylogenies and patterns of diversification to island ages and connectivity (Carson & Clague 1995; Wagner 1995; Roderick *et al.* 2012). The enhanced resolution of molecular techniques is now allowing for comprehensive investigations within islands (Croucher *et al.*, 2012) and across fine-scale geographical variation (Roesch Goodman *et al.*, 2012). The breadth and depth of research in the Hawaiian Islands and the history of integrating multiple lines of evidence allow us to formulate contrasting alternative hypotheses that are grounded in theory and precedented in practice.

The Island of Hawai'i contains dramatic environmental diversity with sharp gradients and natural fragmentation resulting from variation in topography, climate and surface geology. This is particularly true of the wet forest habitat on this island, which is found in four naturally distinct regions: Kohala, Windward, Ka'ū and Kona (Fig. 1). Hawaiian wet forests are found only where volcanic substrate age is at least 200 years old (Kitayama *et al.* 1995) and precipitation is typically at least 2500 mm mean annual precipitation (Gagne & Cuddihy 1990); although in Kona, relatively wet summers maintain sufficiently moist conditions year-round to support distinctly wet forest habitat despite lower annual rainfall. Jacobi (1989) mapped existing areas of wet forest on the Island of Hawai'i, and Price *et al.* (2007) estimated the natural extent through a close examination of relationships between mapped vegetation and climate variables. These distinct natural regions differ markedly in area, substrate age and overall connectivity (Table 1). The gaps between regions differ in both distance and environmental conditions,
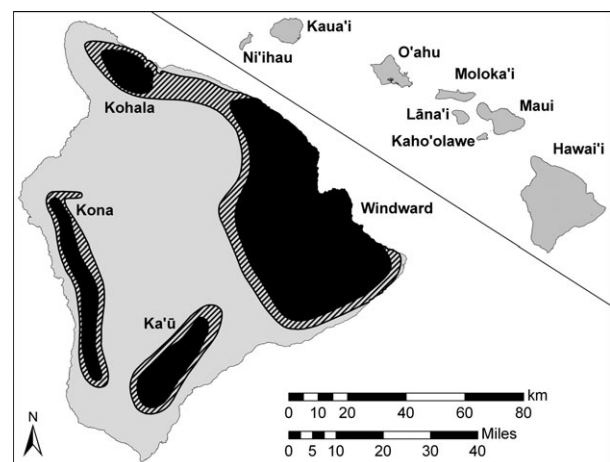
Fig. 1 Wet forest habitat on the Island of Hawai'i. Wet forest habitat regions are shaded black. Hatched areas indicate moist mesic habitats that represent marginal habitat for *Drosophila sproati* and contain many of the plant species found in wet forest.

Table 1 Characteristics of gaps between wet forest regions. Distances were determined from the map of moisture regions by Price *et al.* (2007). Minimum precipitation was estimated from interpolated values by Giambelluca *et al.* (2012). Minimum substrate age was determined from the geologic map by Trusdell *et al.* (2005). Estimated permeability (i.e. the degree to which wet forest organisms may move across gaps) was assessed by considering distance, precipitation and substrate age (see text for explanation)

Habitat gap	Distance (km)	Minimum precipitation (mm)	Minimum substrate age	Estimated permeability
Kohala–Windward	27	2000	Old	High
Kohala–Kona	44	250	Young	Low
Kohala–Ka'ū	79	400	Young	Low
Windward–Kona	51	400	Young	Low
Windward–Ka'ū	23	1500	Young	Medium
Kona–Ka'ū	13	1500	Young	Medium

Table 2 Wet forest regional characteristics. The area of each wet forest region and distance to the nearest wet forest region on another island (Maui) were determined from the map of moisture regions by Price *et al.* (2007). Geologic substrate age (A) as determined by the theoretical timing of the end of the main shield-building stage comes from Clague (1996). Geologic substrate age (B) as determined from the timing of the formation of the major edifice of each volcano comes from Moore & Clague (1992)

Wet Forest Region	Area (km^2)	Distance to Maui Wet Forest (km)	Substrate Age (A) (my, Clague 1996)	Substrate Age (B) (my, Moore & Clague 1992)
Kohala	172	62	0.6	0.4
Windward	2302	103	0.2	0.3
Ka'ū	308	159	0.1	0.1
Kona	300	106	0.4	0.2

including intervening habitat type, climate and substrate age (Table 2). Adding to this current complexity of habitat regions is a dynamic geologic and climatic history. The Island of Hawai'i is a conglomerate of five young volcanoes at the southeast end of the Hawaiian chain, making it the youngest island in a continuous chain of islands that have existed over the last 30 million years (Clague 1996). The five volcanoes that make up the island form a sequence starting with Kohala on the north end of the island and ending with Kīlauea on the southeast (Fig. 2). Evidence from Oahu indicates that recent glacial periods resulted in generally cooler and drier episodes throughout the archipelago, while the changing topography of the Island of Hawai'i probably influenced local orographic- and inversion-dependent moisture (Hotchkiss & Juvik 1999).

Appropriate study systems for an integrated assessment of the general influence of habitat characteristics on ecological and evolutionary processes should share the following criteria: they should be widely studied model systems so that uncommon system-specific characteristics can be identified, they should have comparable spatial distribution in the complex landscape of interest and they should have a variety of known components (e.g. genes or species) from which to observe patterns of local diversity and regional similarity. In addition, they should be as biologically independent as possible, which provides a stronger test of the
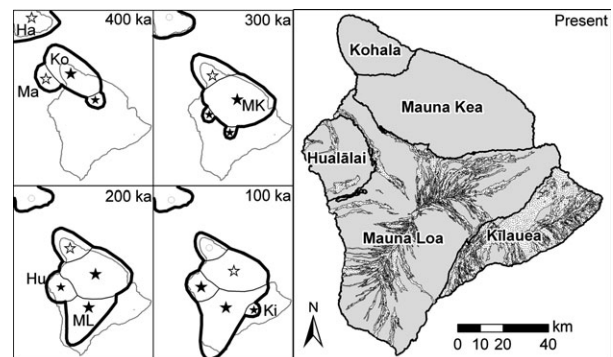
Fig. 2 Geologic history of the Island of Hawai'i. The sequential formation of each of the volcanoes of the island is shown at four points of time following Moore & Clague (1992). Abbreviations indicate each of six volcanoes: Ma = Mahukona (now submerged), Ko = Kohala, MK = Mauna Kea, Hu = Hualālai, ML = Mauna Loa and Ki = Kīlauea. Each volcano goes through distinct stages of emergence, shield building and net subsidence. The result is enlargement of the land area attributable to each, followed by reduction after the end of shield building.

role of habitat characteristics in driving processes than would emerge from a comparison across scales within the same biological system, such as between populations of a species and communities which include that same species. For these reasons, we examined discrete natural wet forest regions through (i) patterns of species

Table 3 Predicted general patterns among mitochondrial and nuclear genetic markers based on demographic history and isolation under the assumption of neutrality. Predicted differential response between markers is due to the lower effective population size of the mitochondrial genome, which is maternally inherited as a nonrecombining single-copy genome. This table does not include the potential for patterns of genetic variation to be influenced by sex-specific migration rates or adaptive sweeps

		Isolated			Connected		
	Genetic marker	Local diversity	Internal distance	Starburst pattern	Local diversity	Internal distance	Starburst pattern
Young (expanding)	Mitochondrial	Low	Low	Strong	Low	High	None
	Nuclear	Low	High	Weak	Low	High	None
Old (constrained)	Mitochondrial	High	High	Strong	High	High	None
	Nuclear	High	High	Strong	High	High	None

composition among communities of wet forest vascular plant for insights into ecological processes and (ii) phylogeographic patterns of an endemic wet forest *Drosophila* species for insights into evolutionary processes.

Hawaiian wet forest vascular plants communities have several attributes that make them ideal for community-level analysis of ecological processes. This habitat type is among the best studied and least impacted by human activity, and floral communities contain a workable number of species, each with its own ecological traits, habitat affinity and history (Cuddihy & Stone 1990). Moreover, given the range of climatic moisture and elevation, many plant species exhibit clear habitat affinities, permitting major community types to be compared directly within and among islands (Price 2004). Compositional patterns of island floras, including those of the Hawaiian Islands (Price 2004), the Canary Islands (Zobel *et al.*, 2011) and the California Channel Islands (Moody 2000), have been useful in testing hypotheses about the role of ecological (immigration and extinction) and evolutionary (speciation) processes in the light of changes to the configuration of islands and habitats over geologic time. Such studies suggest that due to the integration across numerous species, measures of local diversity and regional similarity among plant communities should reflect the general pattern that emerges from ecological processes acting within a complex landscape.

Drosophilid species are widely used in diverse biogeographical settings for population-level analysis of evolutionary processes (Coyne & Orr 2004). With relatively short generation times and high fecundity, which together lead to high-turnover populations, these flies are highly sensitive to evolutionary processes (Coyne & Orr 2004; Markow & O'Grady 2005). In addition, colonization of new habitat appears sometimes to occur from a single fertilized female, such as may be the case in the Hawaiian Islands, where the estimated 800–1000 extant endemic drosophilid species can be traced to a single founding event approximately 26 million years ago (Carson 1982; O'Grady & DeSalle 2008). The

Hawaiian drosophilids have been primarily investigated at the interspecific level but limited population-level analyses have found high levels of divergence within multiple species, including *Drosophila silvestris* (Carson 1982), *D. grimshawi* (Piano *et al.* 1997; Kaneshiro & Kambysellis 1999) and members of the spoon-tarsus subgroup (Lapoint *et al.* 2011). This study examines DNA sequence divergence at two genes among populations of *Drosophila sproati*, a single-island endemic in the *grimshawi* group that is restricted to montane wet forest habitat and is abundant in all of the wet forest regions on the Island of Hawai'i (Markow & O'Grady 2007).[1] Under the assumption of neutrality, variation in demographic history and degree of isolation should result in specific correlated patterns of genetic variation (Avise *et al.* 1987; Zink & Barrowclough 2008; Table 3). Consequently, analysis of local diversity and regional similarity among neutral genetic markers within populations should reflect the general pattern that emerges from neutral evolutionary processes acting within the complex landscape.

Hypotheses

Wet forest habitat on the Island of Hawai'i is currently found only within the four distinct regions that receive approximately 2500 mm of precipitation and contain substrate that is at least 200 year old. However, while precipitation and substrate age are good predictors of habitat type, patterns of local diversity and regional similarity among these regions may be dependent on a variety of other landscape characteristics including

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[1]The larval host plant of *D. sproati* is the common wet forest tree *Cheirodendron trigynum*. This association is likely a factor restricting *D. sproati* populations to wet forest habitat; however, we do not feel that it is a significant confounding issue for this study as both species are abundant in all wet forest regions, and *C. trigynum* is only one of the 200+ vascular plants species found in this habitat type.

habitat patch size, relative isolation and historical climatic conditions. To test the relevance of these characteristics, we developed four biogeographical hypotheses a priori from alternative interpretations of how landscape characteristics drive ecological and evolutionary processes to produce observable patterns. These alternative hypothesis are derived from theoretical arguments and observations from less complex landscapes that have not necessarily been corroborated at this scale of biogeographical complexity. The relevant landscape characteristics, justifications and associated predictions of local diversity and regional similarity are summarized in Table 4, and a more complete description of each hypothesis can be found in the supplemental materials. This tested set of hypotheses does not include adaptive hypotheses that might be derived from the variation in rainfall among regions (Table 1) or observed changes in soil nutrients with substrate age (Vitousek *et al.* 1995). Such adaptive hypotheses cannot be rigorously tested through our analysis of landscape-dependent neutral processes that are expected to produce analogous patterns across biological scales. However, adaptive divergence among *D. sproati* populations would not easily explain patterns among the neutral genetic markers. Additionally, relative to the species area trend of the archipelago, this young island supports wet forest communities that are comparatively depauperate (Price 2004). According to the island immaturity–speciation pulse model (Whitaker *et al.* 2007), such young islands are dominated by immigration processes, which in turn are strongly influenced by area and proximity to sources. Within the Island of Hawai'i, any differences in environmental conditions among wet forest regions are potentially less important than landscape characteristics. Therefore, selective filters to plant community assembly may be overridden by neutral ecological processes. For all hypotheses, human-induced fragmentation of wet forests is considered to be too recent to strongly influence the genetic diversity of *D. sproati* or the species diversity of the vascular plant communities (Cuddihy & Stone 1990).

Methods

Vascular plant community analysis

To determine which plant species occur within each region, we drew from a database of angiosperm distributions (Price 2004) with additional data for ferns (Palmer 2003) to represent all vascular plants. These data come from numerous sources, including herbarium specimens, species lists, historical accounts and field records from numerous biologists. Data include (separately) presence in different climate zones and presence within different regions of the island (separate volcanoes, plus Mauna Loa divided into four slopes). Given the paucity of records in some areas, and due to the intensity of human impacts (Cuddihy & Stone 1990), the presence of some species in a given habitat region is uncertain and they may no longer occur there. In such cases, the only record placing a species within a given part of the island (for example the Kohala Mountains) may not have locality data specific enough to place it within that wet forest habitat region. We therefore followed Price (2004) and considered a species to inhabit a wet forest habitat region if it was recorded from any of the volcanoes that make up the region and is known to occur in wet forest elsewhere within its range. While this method may lead to an overestimation of the occurrence of a few species, the alternative of rejecting records that credibly represent a presence in a given habitat region would probably result in a greater rate of error. This method therefore includes many species that occur in wet forest but whose ranges extend to drier habitats.

We analysed species composition in two ways. First, we considered all species that probably occurred within each given wet forest habitat region. We also analysed composition of species that are restricted to wet forest. While this is a much smaller number of species, these may be more indicative of processes specific to wet forest (as opposed to slightly drier mesic habitats that have a different configuration and presumably different history). We performed analyses separately for all wet forest species and for species restricted to wet forest (henceforth referred to as 'all' and 'restricted'). We assessed the total number of species present in each wet forest habitat region and the number endemic to each region. We calculated the Jaccard's index for each pair of regions. This index represents the number of shared species divided by the number of species collectively contained by both regions ($|A \cap B| / |A \cup B|$), where a value of 1 represents identical composition, and a value of zero indicates no species in common. This index is somewhat sensitive to difference in species number, such that uneven richness results in a lower value. If one region's species composition is a complete subset of the other, the index will be less than one, highlighting the fact that there are species in one not contained in the other. However, Shi (1993) found that among commonly used ecological indices, Jaccard's proved to be the least influenced by differences in the size of species pools; in other indices (such as Sørenson's), disparity in species pool size translates to low similarity values. Consequently Jaccard's index is widely used in examinations of compositional similarity (Nekola & White 1999). For both data sets, we then ran a cluster analysis using a nearest neighbour algorithm

based on Jaccard's index. We also gathered data on the species composition of East Maui (the closest wet forest region on another island). This allowed us to assess relationships between the floristic composition of the most likely source of colonist species and that of various regions of the Island of Hawai'i. Results of the cluster analysis were displayed as dendrograms, which indicate overall relationships among all regions.

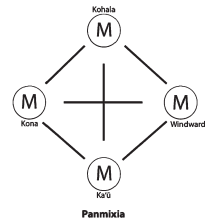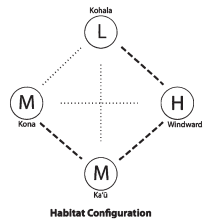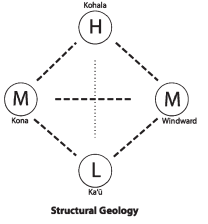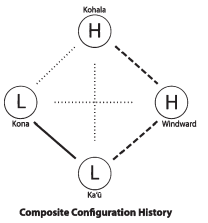### Phylogeographic patterns in Drosophila sproati

A minimum of 33 *Drosophila sproati* individuals were collected from single locations in northeast Ka'ū, Central Kohala and South Kona and from three locations spanning the larger Windward region. All individuals were captured live in vials while attracted to fermented banana bait spread onto sponges that were hung from trees at 1–2 m off the ground. Captured flies were placed in 95% ethanol and stored at −20 °C. DNA was extracted from the flies using a nondestructive soak technique modified from Rowley *et al.* (2007) in combination with reagents from Qiagen DNeasy extraction kits (Qiagen). Postextraction specimens were returned to 95% ethanol and placed in long-term storage at −20 °C, as was all extracted DNA. The cytochrome oxidase II (COII) region of the mitochondrial genome was amplified using the primer pair 'A-tLEUmod' (5′ ATGGCAGATTAGTGCAACGG3′) and 'B-tLYS (5′ GTTTAAGAGACCAGTACTTG3′) and the following thermocycle conditions: 7 min at 94 °C followed by 35 cycles of 1 min at 94 °C, 45 s at 57 °C and 30 s at 72 °C and a final incubation of 5 min at 72 °C (Liu & Beckenbach 1992). The nuclear yolk protein 1 (YP1) gene on the X chromosome was amplified with the custom primers YP1F (5′ AAGCCATCGCAATGGTTATC3′) and YP1R (5′ ATTTTGGCCAATCAAATGGA3′) and the same thermocycle conditions but with an annealing temperature of 55 °C. All PCR products were purified using a Qiagen PCR Purification kit (Qiagen) or through enzymatic purification, which consisted of the addition of 2.5 U exonuclease I, 1 U shrimp alkaline phosphatase (SAP) and 1 μL SAP reaction buffer to each 15–25 μL of PCR product and an additional thermocycle of 30 min at 37 °C followed by 20 min at 80 °C. All purified PCR products were sequenced at the University of Hawai'i at Manoa at the Advanced Studies in Genomics, Proteomics and Bioinformatics laboratory or the Genomics Core Facility in the affiliated John A. Burns School of Medicine. All sequence returns were visually aligned and edited using Sequencher version 4.9 (Gene Codes Corp.). Both fragments were sequenced in both directions, but the 751-base pair COII fragment was trimmed to 570 bp for analysis to exclude consistently low-confidence base reads and the 760-base pair YP1

fragment was trimmed to a 500-bp portion containing an 82-bp intron. As the YP1 gene is found on the X chromosome, unique alleles were first identified in males and homozygous females, and the alleles in heterozygous females were characterized accordingly. Females that could not be unambiguously genotyped, which was rare, were not included in the analysis. Allele frequencies were subsequently calculated as the total alleles present in a population with no distinction made between males and females. In all cases, the sampled locations were taken as representative of the larger natural wet forest regions with the multiple sites within the Windward region collapsed into a single population for all analyses.

TCS version 1.21 was used to construct haplotype and allele networks under statistical parsimony (Clement *et al.* 2000). PopTools was used to calculate corrected haplotype and allele counts for each population, using 100 bootstrapped replicates at $n = 30$ for COII and $n = 40$ for YP1 (Hood 2010). Arlequin version 3.1 was used to calculate the mean number of pairwise differences, Tajima's D and Fu's F_S and to perform mismatch distribution analysis, all at 1000 bootstrapped replicates (Excoffier *et al.* 2005). Tajima's D and Fu's F_S are both neutrality tests, which can be used to infer historical population dynamics with significantly negative values indicating population expansion (Tajima 1989; Fu & Li 1993). Mismatch distribution analysis compares observed distributions of pairwise differences against distributions predicted by demographic or spatial expansion simulations, with significant sum of square deviations and Harpending's raggedness indices indicating a stable or decreasing population over time (Slatkin & Hudson 1991; Rogers & Harpending 1992). Analysis of molecular variance (AMOVA) could not be used for direct hypothesis testing due to the small number of possible permutations (Fitzpatrick 2009). However, AMOVA results were calculated in Arlequin for the clear cladistic division seen in the *D. sproati* COII results to give a measure of population versus clade variation for this single grouping.

The COII and YP1 gene networks were left unrooted due to the complex genetic history of *D. sproati*. Phylogenetic work using nuclear DNA sequences has shown that *D. sproati* does not have an extant sister species on Maui, but diverged from a common ancestor with the species pair *D. orphnopeza* (Maui/Molokai) + *D. murphyi* (Hawai'i) prior to the origin of the Island of Hawai'i (D.K. Magnacca & K. Price, in preparation; Fig. 3). Moreover, the mtDNA mutation rate appears to be elevated in *D. sproati* compared to its closest relatives, causing it to group with more distantly related species such as *D. orthofascia* when mtDNA sequences from all species in the *orphnopeza* subgroup are analysed (D.K. Magnacca & K. Price, in preparation).

Table 4 Summary of the landscape characteristics that were used to develop the alternative biogeographical hypotheses of wet forest biota on the Island of Hawai'i, the corresponding theoretical and observational justifications and the predicted patterns of local diversity and regional similarity. Diversity within each region is noted as high (H), medium (M) and low (L). Similarity between regions is noted as high (solid line), medium (dashed line) and low (dotted line). More complete descriptions of each hypothesis are given in the supplementary materials

| Hypothesis	Relevant landscape characteristics driving observable patterns	Theoretical/observational justifications	Predicted pattern of local diversity and regional similarity
Panmixia	No relevant landscape characteristics at this geographical scale	Isolation of Hawaiian archipelago favours species with high dispersal ability (Carlquist 1974) Observed lack of differentiated within some groups (Price & Wagner 2004; Nitta & O'Grady 2008) and species (Magnacca & Brown 2010; Adamski *et al.* 2012)	
Habitat Configuration	Habitat patch sizes Intervening distances Habitat gap characteristics	Theory of Island Biogeography (MacArthur & Wilson 1967) in community ecology and observations of analogous patterns in phylogeography (Nei 1972; Avise *et al.* 1987) Observed patterns of diversity and relatedness among island biota within the Hawaiian archipelago (Funk & Wagner 1995; Price 2004)	
Structural Geology	Ages of geologic structures	'Progression rule' describing directional colonization of younger habitats from adjacent older habitats, such as in hotspot island chains (Hennig 1966; Wagner 1995; Cowie & Holland 2008)	
Composite Configuration History	Habitat patch sizes Intervening distances Habitat gap characteristics Ages of geologic structures Regional climatic history Interactions of the above	Historical habitat configuration is more relevant to ecological and evolutionary processes than the current configuration In a dynamic landscape, the current habitat configuration may not represent historical configuration The age of a habitat patch is dependent on the interaction of substrate age and regional climatic conditions and neither alone is a sufficient proxy of habitat age Spatial and temporal landscape characteristics are always interacting to drive ecological and evolutionary processes	

Results

Vascular plant community analysis

Of the 512 species of native vascular plants known from the Island of Hawai'i, 337 species are known to occur in wet forest, 61 of which are restricted to wet forest. Of those occurring in wet forest, 54 species are endemic to the Island of Hawai'i. Analysis of all species found in wet forest reveals a clear pattern of species richness among habitat regions: Windward (294) and Kohala (252) have more species than Ka'ū (224) and Kona (212). The number of regional endemics follows nearly the same rank order: Windward (7), Kohala (6), Kona (1) and Ka'ū (0). Species restricted to wet forest exhibit a similar pattern, but with a greater range: Windward (48) and Kohala (38) have more species than Ka'ū (20) and Kona (10). The pattern among regional endemics

restricted to wet forests reflects that of other analyses: Windward (4), Kohala (4), Kona (1) and Ka'ū (0).

Jaccard similarity indices for all species indicate that Kohala contains the most distinct plant community on
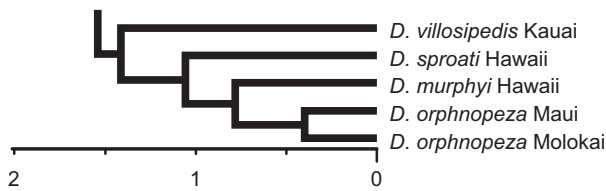
Fig. 3 Phylogram of the *Drosophila sproati* species complex, based on Bayesian analysis of five nuclear genes (EF-1γ, Yp2, Fz4, Pgi and Gpdh; D.K. Magnacca & K. Price, in preparation). Scale bar units are millions of years before present.
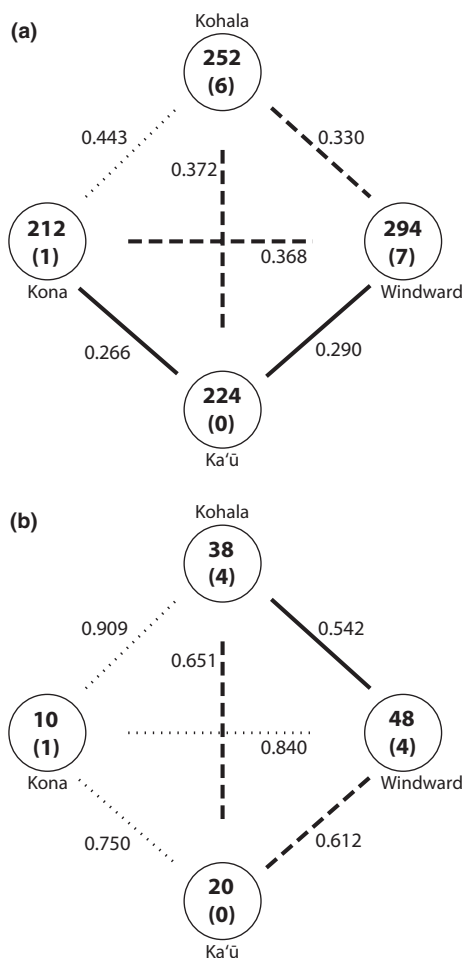

Fig. 4 Summary of regional diversity and similarity patterns among (a) all vascular plant species found in wet forest habitat and (b) vascular plant species restricted to wet forest habitat. The number within each circle is the total number of vascular plant species found within each region, and the number in parentheses is the number of regional endemics. Regional similarity was measured as Jaccard's index.

the island, with Kona and Ka'ū containing the most similar communities (Fig. 4a). This contrasts somewhat with the pattern for plants restricted to wet forest where Kona is dissimilar to the other regions, which are all moderately similar (Fig. 4b). The dendrogram for all species shows the greatest difference in species composition is between East Maui and all Island of Hawai'i regions, followed by Kohala and the other three regions (Fig. 5a). Ka'ū and Kona are the least differentiated among all regions. This contrasts with the dendrogram for species restricted to wet forest, which identifies Kona as the most strongly differentiated from all other regions, followed by East Maui, and identifies Windward and Kohala as the least differentiated regions (Fig. 5b).

Phylogeographic patterns in Drosophila sproati

The mitochondrial COII region contained 31 polymorphic sites among 255 *D. sproati* individuals, resulting in 31 unique haplotypes with a maximum distance of nine base pair changes between haplotypes. Intrapopulation characteristics differed markedly among the populations with genetic diversity higher in Kohala and Windward than in Ka'ū and Kona (Table 5). Kohala and Windward share dominant central haplotypes as do Kona and Ka'u but only Kona displays a tight starburst pattern, while Ka'ū shares a haplotype with the Kohala/Windward cluster (Fig. 6). Analysis of genetic differentiation through pairwise F_{ST} values quantifies this strong divide between northeast and southwest populations and identifies Ka'ū as the apparent intermediary, while Kohala and Kona are the most differentiated (Fig. 7a). Tajima's *D* and Fu's F_S values were significant
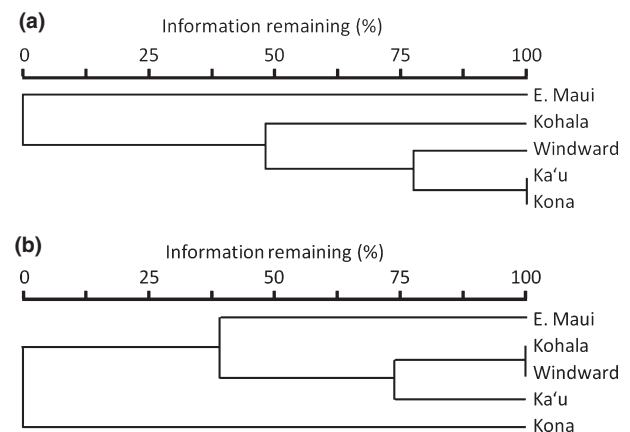

Fig. 5 Dendrograms based on hierarchical cluster analysis, using nearest neighbour linkage of Jaccard's similarity index. (a) Dendrogram for analysis of all species occurring in wet forest. (b) Dendrogram for analysis of species restricted to wet forest.

Table 5 Regional genetic diversity within a 570-bp fragment of the COII gene and the 500-bp fragment of the YP1 gene

	Population	n	Male/Female	N	Corrected N	SD/95% C.I.	Maximum pairwise distance	Mean # pairwise differences
COII	Kohala	44	24/20	10	6.0	1.5/0.3	5	0.87 ± 0.62
	Windward	139	70/69	16	6.3	1.7/0.3	5	0.88 ± 0.62
	Ka'ū	33	16/17	5	3.9	0.9/0.2	4	1.12 ± 0.75
	Kona	39	19/20	6	4.4	1.0/0.2	2	0.40 ± 0.38
YP1	Kohala	62	24/19	11	7.3	1.4/0.3	2	0.70 ± 0.54
	Windward	198	68/65	13	6.0	1.4/0.3	3	0.59 ± 0.48
	Ka'ū	47	17/15	9	7.0	1.1/0.2	3	0.92 ± 0.65
	Kona	57	19/19	6	5.3	0.7/0.1	3	0.88 ± 0.62

n is the number of sequenced haplotypes (one per individual) and allele copies (one per male, two per female). N is the number of unique haplotypes/alleles found within each population and corrected N is the mean number of unique haplotypes found after 100 bootstrapped resamplings at $n = 30$ for COII and $n = 40$ for YP1.

Kohala (44)   Windward (139)   Ka'ū (33)   Kona (39)   Missing haplotype
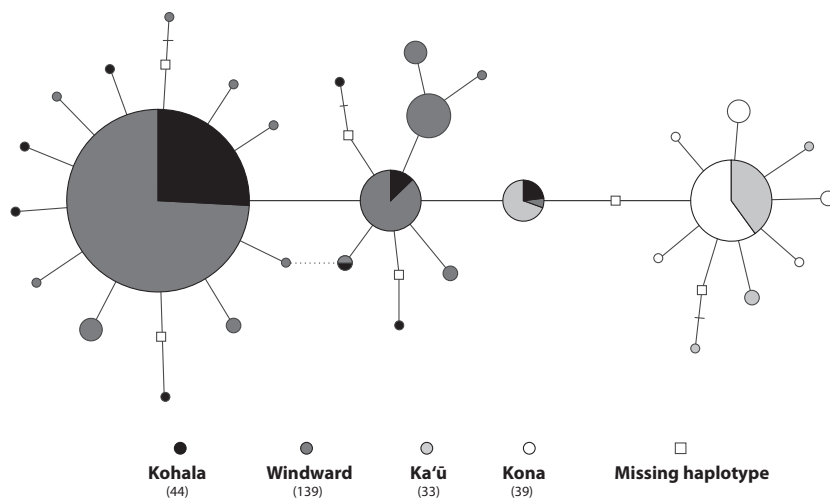
Fig. 6 Minimum-spanning network of *Drosophila sproati* COII haplotypes. Each circle represents a unique haplotype with the size proportional to the frequency of that haplotype out of the total. Each connection represents a single base pair change and those changes that resulted in an amino acid change are noted with a bar.

and negative for all populations except for Ka'ū, and only Kohala deviated significantly from rapid expansion population models in mismatch distribution analysis (Table 6). Grouping Kohala with Windward and Ka'ū with Kona for analysis of molecular variance (AMOVA) was highly explanatory as this grouping accounted for 76.96% of the species-wide genetic diversity with only 1.51% due to variation among regions within these groups (Table 7).

The nuclear YP1 region contained a total of 18 polymorphic sites, seven of which were in the intron and 21 unique alleles among the 346 allele copies sequenced from 225 *D. sproati* individuals. All populations were dominated by a single shared allele, and Kohala, Windward and Ka'ū showed similar internal characteristics, while Kona was relatively depauperate of alleles and contained a widespread but otherwise rare (<5%) allele in relatively high (>20%) abundance (Table 5; supplementary materials). Pairwise F_{ST} analysis found the

Kona population to be significantly differentiated from all other populations and no significant differentiation between Kohala, Windward and Ka'ū (Fig. 7b). Tajima's D and Fu's F_S values were significant and negative for all populations except for Kona, while Ka'ū alone showed significant deviation from rapid expansion models when measured as sum of squares deviation from a demographic expansion model (Table 6). The species-wide allelic minimum-spanning network shows a single starburst pattern with a maximum distance of four base pair changes between extreme alleles and no clear geographical clades (supplementary materials).

Discussion

The analysis of all vascular plant species found within wet forests on the Island of Hawai'i showed strong differences in local diversity among the regional communities, with Windward containing the most diverse

(a)

(b)
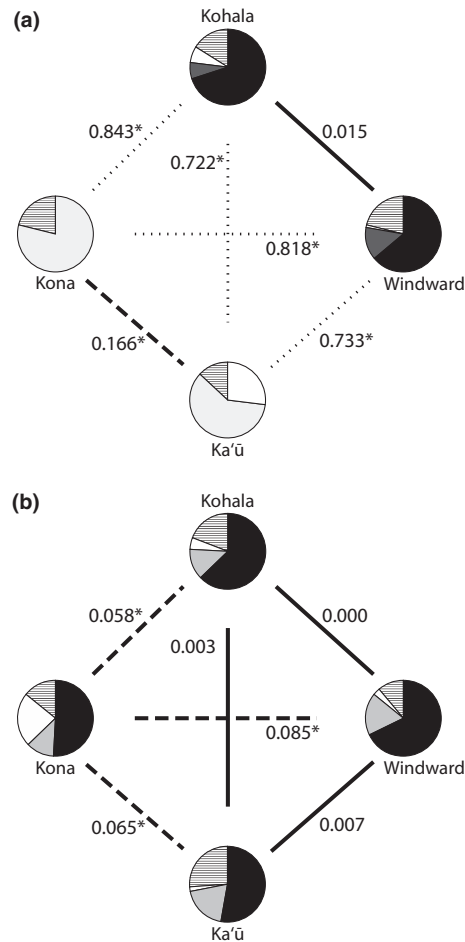
Fig. 7 Genetic differentiation among *Drosophila sproati* populations at the (a) COII and (b) YP1 regions. Regional proportions of all haplotypes/alleles that are found at >10% within at least one population are colour-coded among the populations within each figure. The hatched areas are the combined proportion of all other haplotypes/alleles. Pairwise F_{ST} values are expressed on the lines connecting the regions with significant differentiation noted with an asterisk and represented by a dotted (high F_{ST} value) or dashed (intermediate F_{ST} value) line. Insignificant differentiation is represented by a solid line.

plant community followed by Kohala, Ka'ū and Kona. This ranking was reinforced through analysis of regionally endemic species, which also identified an apparent metagrouping with low levels of endemism in the leeward regions (Kona and Ka'ū) and higher levels in the windward regions (Kohala and Windward). This leeward/windward divide was more distinctive when the analysis of endemic species was limited to those restricted to wet forests, which also showed the same rank order of local diversity. As the accumulation of endemic species and wet forest restricted colonists is expected to take more time than the colonization of species that can tolerate more mesic conditions, these results suggest that the plant communities in Ka'ū and Kona are younger than those in Kohala and Windward. Jaccard similarity indices of both all species and wet forest restricted species identified Kohala and Kona as consistently containing the most distinct communities, suggesting that Windward–Ka'ū is the point of contact between the leeward and windward groups. The analysis of all species identified the community in Kohala as relatively distinct from all other regional communities, while the analysis of wet forest restricted species identified Kona as highly distinct from all other regional communities.

The analysis of the COII gene in *Drosophila sproati* populations also showed a strong cladistic divide between Kohala–Windward and Kona–Ka'ū, and the minimum-spanning haplotype network further suggests that Windward–Ka'ū is the point of contact between these two clades. The haplotype network also identified the Kona *D. sproati* population as being younger and more isolated than the Ka'ū population. Unlike the plant community results, Kohala and Windward populations were not significantly differentiated at the COII gene, while Kona and Ka'ū populations were. Analysis of the YP1 gene identified Kona as containing a uniquely differentiated population due to low genetic diversity, a potential result of recent colonization, but

Table 6 Results of neutrality tests and mismatch distribution analysis of the COII and YP1 regions in *Drosophila sproati*

	Population	D	F_S	SSD—Demographic	SSD—Spatial	Raggedness—Both
COII	Kohala	−1.95*	−6.57*	0.35	0*	0.11*
	Windward	−1.79*	−12.41*	0*	0*	0.05*
	Ka'ū	−0.68	−0.23	0.07*	0.05*	0.28*
	Kona	−1.69*	−4.39*	0*	0*	0.18*
YP1	Kohala	−1.85*	−8.71*	0.01	0.01	0.15
	Windward	−1.73*	−12.14*	0.01	0.01	0.14
	Ka'ū	−1.53*	−5.98*	0.01*	0.01	0.14
	Kona	−0.44	−1.28	0.02	0.02	0.15

Asterisks mark results that indicate historically expanding populations, measured as either significant negative Tajima's D ($P < 0.05$) and Fu's F_S ($P < 0.05$) values or insignificant ($P > 0.05$) deviation, measured as sum of squares (SSD) and raggedness, from a rapid expansion model.

Table 7 Analysis of Molecular Variance among the COII haplotypes when grouping Kohala with Windward and Ka'ū with Kona. The degrees of freedom (d.f.) and sum of squares (s.s.) are listed for each component analysis. The *P*-value was calculated at 1000 permutations

	% Variance (*P*-value)		
Grouping	Among groups	Among populations within groups	Within populations
Kohala + Windward	76.96% (<0.001)	1.51% (<0.001)	21.53% (<0.001)
Ka'ū + Kona	d.f. = 1	d.f. = 2	d.f. = 251
	s.s. = 157.3	s.s. = 3.9	s.s. = 105.4

otherwise did not show any regional differentiation. The different patterns observed at the COII and YP1 markers are likely due to the characteristics of the genes themselves, with the maternally inherited single-copy mitochondrial gene expected to be more responsive to the population bottleneck/founding event that would have occurred in the initial colonization of Ka'ū from Windward (Zink & Barrowclough 2008).

The two biological scales analysed in this study, communities of vascular plants and genes within *D. sproati* populations, displayed slightly different patterns of local diversity and regional similarity. For one, the Kohala and Windward populations of *D. sproati* were highly similar at both sampled genes, while the vascular plant communities in these regions have moderately different species assemblages. This disparity probably reflects the difference between these analyses; with the assumption of neutrality, the sampled genes of the high-turnover fly populations are expected to not differentiate under even relatively low migration rates and to come quickly to equilibrium with recent high migration (Slatkin 1987). In contrast, the vascular plant communities, which are made up of individual species with a wide range of relevant system-specific characteristics such as dispersal ability, may be expected to be less responsive as a whole and retain regional distinctiveness longer than the neutral markers in *D. sproati*.

More striking than these differences is the strong similarity of the patterns of local diversity and regional similarity that were observed within vascular plant communities and genes within *D. sproati* populations (Fig. 8). With the exception of the YP1 gene, which showed low overall resolution, local diversity was found to be highest in the large Windward region across biological scales, followed closely by the much smaller Kohala region, with Ka'ū and Kona being markedly less diverse than both. Regional similarity showed a strong distinction between Kohala–Windward and Ka'ū–Kona with Windward–Ka'ū being much more similar than Kohala–Kona.

This corroborated general pattern of local diversity and regional similarity provides a compelling test of the
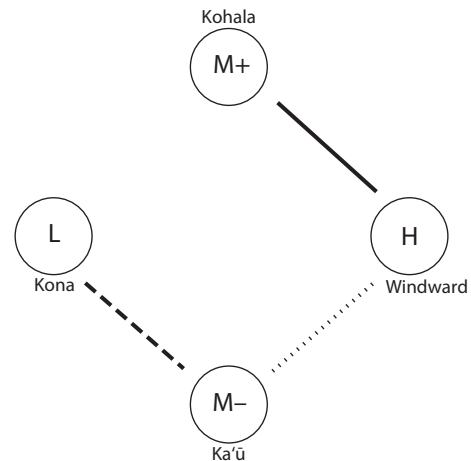
Fig. 8 Generalized pattern of regional diversity and similarity found among genes in *Drosophila sproati* and species within wet forest vascular plant communities. Diversity within each region is noted as high (H), medium (M+, M−) and low (L). Similarity between regions is noted as high (solid line), medium (dashed line) and low (dotted line). Regional connections with little support (very low similarity) have been removed.

a priori hypotheses. Most obviously, it indicates that Panmixia is not an appropriate general biogeographical model of the Island of Hawai'i and should not be assumed unless there are system-specific reasons to expect it, which might include high dispersibility or very recent colonization. While interpreting current landscape characteristics is an improvement and can be made from relatively simple geographical knowledge, the habitat configuration model also did not accurately predict the observed general pattern. For example, it cannot account for the high diversity and endemism in Kohala or the dramatic break between Windward and Ka'ū. Again, the predicted pattern may be found in some biological systems on the island, such as recently arrived species with intermediate dispersal ability. However, in general, such a model should not be expected to produce reliable predictions unless regional habitat characteristics and configuration have been relatively stable within a time span relevant to the system of

interest. Similarly, the predictive value of a model based solely on geologic history may be limited to relatively simple geographical systems and scales, such as between islands in a hot-spot archipelago (Wagner 1995), but may be inappropriate within the same islands. In this study, the Structural Geology model did not predict the striking difference between Kona and Windward regions as both contain substrates of similar age, nor the higher diversity in Ka'ū compared with Kona considering the younger substrate of the former. Given the increasing ability to use multiple lines of evidence to infer historical environmental conditions and produce reliable general climate models, single measures such as substrate age should not be relied upon for historical interpretations unless the situation specifically allows for it.

The similarity of the observed general pattern to the predictions made by the Composite Configuration History hypothesis supports a detailed biogeographical model of wet forest habitat in the complex landscape of the Island of Hawai'i. In this model, ecological and evolutionary processes acting on wet forest biota are influenced by the dynamic interaction of spatial, temporal and climatic factors as described by this hypothesis. However, the Composite hypothesis did not predict the observed significant differentiation between Ka'ū and Kona wet forest regions, which indicates that the most holistic hypothesis failed to take into account some important element(s). This differentiation in plant communities may best be explained by incorporating the historical dynamics of the slightly drier 'moist mesic' habitat type (hatched area of Fig. 1, Price *et al.* 2007), which contains many of the species that are found in but not restricted to wet forests. As the volcanoes of Mauna Kea and Mauna Loa developed in size, the associated increasing convection-based moisture in the leeward regions (Kona and Ka'ū) would have resulted in moist mesic habitat developing before and more extensively than wet forest habitat. The described habitat gaps, however, would have remained relatively constant due to the active intervening lava flows separating Kona, Ka'ū and Windward and the constantly arid conditions between Kona and Kohala. As moisture increased, the developing Ka'ū wet forests would have been colonized from both the existing moist mesic habitat in the region and the adjacent established wet forests in the Windward region. In contrast, the developing wet forests in Kona would have been colonized primarily from the local moist mesic habitat and only secondarily, via Ka'ū, from established Windward wet forests. This interpretation is consistent with the observation that Kona contains only half as many plant species that are restricted to wet forests as Ka'ū, while the total number of species found in wet forests are more similar. An alternative explanation for the differentiation between Kona and Ka'ū is that the lower precipitation in Kona results in drier conditions that create a selective filter to migration and colonization. However, Kona wet forests are structurally similar to other wet forest regions, as they support high abundance of both tree ferns and epiphytes and comparatively low abundance of species typically dominant in moist mesic habitat such as Koa (*Acacia koa*) and Olopua (*Nestegis sandwicensis*) (Jacobi 1989). Such selective pressure would also not explain the observed patterns in the two independent neutral markers within the *D. sproati* populations, which are expected to respond primarily to neutral processes such as drift and gene flow. The observed patterns of low diversity and significant differentiation from Ka'ū, as well as the starburst pattern in the COII gene, are, however, well explained by a recent colonization of Kona by *D. sproati* and limited gene flow across the young substrate in the Ka'ū–Kona habitat gap. Moist mesic habitat may also serve as a limited migratory corridor for *D. sproati*, which, through associated gene flow, could account for the low differentiation between the Windward and Kohala populations.

The incorporation of this more nuanced historical habitat analysis into the Composite Configuration History hypothesis posits an explicit and corroborated general biogeographical model for wet forest habitat on the Island of Hawai'i. Comparison against this model should identify nonrepresentational systems and may help to determine important system-specific characteristics that would account for alternative patterns. For example, extensive morphological, behavioural and genetic investigations of *Drosophila silvestris*, another endemic wet forest picture-winged *Drosophila*, indicate that Kona contains the most ancestral *D. silvestris* population on the island and subsequent colonization of wet forest regions followed a southern route into Ka'ū and a northern route into Kohala and Windward with no apparent gene flow across the Windward–Ka'ū gap (Carson 1982; Craddock & Carson 1989). Initial colonization of Kona wet forests rather than those on Kohala is possible, while the alternative pattern of regional differentiation may have been driven in part by the host plant characteristics of this species. *Drosophila silvestris* currently oviposits in both *Cheirodendron trigynum*, the ubiquitous wet forest tree also used by *D. sproati*, and the 11 species in the genus *Clermontia* found on the island (Magnacca *et al.* 2008). As *D. planitibia* and *D. differens*, the Maui and Moloka'i relatives of *D. silvestris*, are limited to *Clermontia* species as a host plant, the early *D. silvestris* populations were probably closely linked with the patchy distribution of *Clermontia* species on the island, a factor that has been shown to correspond to population differentiation in other *Drosophila* species (Shoemaker & Jaenike 1997). In addition,

local adaptation to specific *Clermontia* species could have resulted in more complex population divergence and the observed unusual patterns of population differentiation. However, given the striking contrast to the corroborated general model, *D. silvestris* populations should be reanalysed using higher resolution genetic techniques to verify the observed alternative picture of regional connectivity.

This combined investigation of landscape patterns among vascular plant communities and *D. sproati* population genetics presents an improved method for developing corroborated biogeographical models within complex landscapes. The resulting corroborated model of ecological and evolutionary processes in a complex landscape is a dramatic improvement over models that are derived from simple proxies of habitat characteristics or single biological systems, such as *D. silvestris*. In addition, in well-studied biogeographical systems, the accumulation of diverse knowledge, particularly with the advent of genetic techniques, has led to increased interest in synthesizing observable patterns to draw general biogeographical conclusions. Examples include the Philippine Islands (Jones & Kennedy 2008), the Canary Islands (Juan *et al.* 2000), the Galapagos Islands (Parent *et al.* 2008) and the West Indies (Ricklefs & Bermingham 2008). However, while the goal of such syntheses is a general model of relevant ecological and evolutionary processes, the common approach is to summarize existing studies. As such, any conclusion may be influenced by the research bias within the given biogeographical system. This bias can be limited through the careful selection of appropriate biological study systems for integrated analysis, such as *D. sproati* over *D. silvestris* due to host plant considerations and wet forest vascular plants over passerine birds due to sampling and recent extinction concerns (James & Olson 1991; Magnacca *et al.* 2008).

This multiscale and hypothesis-driven approach should also prove valuable in landscapes where little is known. There is now sufficient global high-resolution spatial data and coherent models of historic geologic and climatic conditions to develop composite hypotheses in arguably any geographical setting. Such composite models can, with care, be subsequently refined using seemingly disparate systems prior to the beginning of an investigation. Specific composite prediction will also depend on system-specific characteristics, for example panmixia may be predicted under certain conditions for certain systems, but the potential to explicitly integrate multiple lines of evidence and develop informed predictions should help provide direction to novel biogeographical research.

Finally, this study illustrates the need to interpret landscapes as complex and dynamic biogeographical systems and to use this complexity to inform predictions at any biological scale. Many important landscape characteristics, including those related to habitat size and habitat age, have been identified from studying relatively simple biogeographical systems such as island archipelagos. However, these ideas cannot be directly applied in isolation across biological systems and scales due to the potential for interacting processes. The use of discrete biogeographical hypotheses demands rigorous articulation of landscape complexity and when formulated prior to observation allows for testing of alternative models of locally relevant processes.

Conclusion

Explicit biogeographical hypotheses allow for rigorous testing of locally relevant evolutionary and ecological processes in complex landscape through the comparison of predicted patterns against those observed in biological systems. Recent theoretical developments have brought community ecology and population genetics together, allowing for hypothesis testing across analogous but independent biological scales. This study uses a particularly well-studied biogeographical setting, the Island of Hawai'i, to exemplify how alternative models can be created a priori from multiple biogeographical insights, and how they can be tested, corroborated and improved upon through diverse analysis of patterns across biological scales. The results clearly show the advantages of biogeographical models that integrate information from ecology, geology, climatology and other fields over those that rely on singular lines of evidence. This integrated and hypothesis-based method can both guide initial research in complex landscapes and summarize existing findings into comprehensive relationships between landscape characteristics, fundamental processes and observable patterns in biological systems.

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Data accessibility

DNA sequences: Genbank accessions JX455020-JX455050 (COII); JX454999-JX455019 (YP1). Individual *Drosophila sproati* collection information and wet forest vascular plant species list: DRYAD entry doi:10.5061/dryad. cn946.

Supporting information

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

Table S1 Observed frequencies of COII haplotypes in *Drosophila sproati* on the Island of Hawaii, measured as total count and % of regional population.

Table S2 Observed frequencies of YP1 alleles in *Drosophila sproati* on the Island of Hawaii, measured as total count and % of regional population.

Table S3 Summary of *Drosophila sproati* collection sites on the Island of Hawaii.

Fig. S1 Minimum spanning network of *Drosophila sproati* YP1 alleles.