



Historical biogeography and ecological opportunity in the adaptive radiation of native Hawaiian leafhoppers (Cicadellidae: *Nesophrosyne*)

Gordon M. Bennett* and Patrick M. O'Grady

University of California, Department of Environmental Science, Policy and Management, Berkeley, CA, 94720, USA

ABSTRACT

Aim Our aims were to infer the roles of ecology and geology in the adaptive radiation, historical biogeography, and diversification dynamics of the native Hawaiian leafhoppers (Hemiptera: Cicadellidae: *Nesophrosyne*).

Location The Hawaiian Archipelago.

Methods A six gene molecular data set for 191 *Nesophrosyne* species was used to reconstruct relationships in absolute time with relaxed Bayesian methods. Dated phylogenetic hypotheses were used to reconstruct historical biogeography and host plant associations. Phylogenetic clustering methods were implemented to examine the structuring of *Nesophrosyne* species according to island. Lineage diversification dynamics were inferred using the γ -statistic and birth-death likelihood methods.

Results *Nesophrosyne* split from a common ancestor with its sister genus 4.5 million years ago (Ma), with a basal divergence in Hawaii 3.2 Ma. Historical biogeographical reconstructions reveal a Kauai origin for the genus and complex island colonization patterns. Ancestral host plant associations reconstruct Urticaceae and Rubiaceae at the root, with host transitions resulting in host-specific clades. Only species endemic to Kauai are significantly clustered with respect to the whole phylogeny; however, species show significant terminal level clustering for the four main high islands. Diversification rates in *Nesophrosyne* show an initial burst in net speciation rate with a subsequent decline.

Main conclusions *Nesophrosyne* colonized the Hawaiian Islands after the formation of Kauai. Host plant arrival times and insect–insect competition may have been important in shaping diversification patterns. The plant families Urticaceae and Rubiaceae played critical roles in the early diversification of *Nesophrosyne*. Island geography imposed significant barriers to gene flow, leading to extensive allopatric speciation and intra-island diversification. A high initial net speciation rate was associated with host plant transitions, and the formation and establishment of *Nesophrosyne* on multi-volcano islands (e.g. Oahu and Maui Nui). Net diversification rates exhibit a diversity-dependent decline, corresponding to the end phase of island formation.

Keywords

Adaptive radiation, Auchenorrhyncha, biogeography, diversification rates, ecological opportunity, Hawaii, island biogeography, island evolution, leafhoppers, lineages through time, *Nesophrosyne*.

*Correspondence: Gordon M. Bennett, University of California, Berkeley, Department of Environmental Science, Policy and Management, 137 Mulford Hall #3114 Berkeley, CA 94720, USA. E-mail: gbennett@berkeley.edu

INTRODUCTION

Adaptation to novel ecological niches and non-adaptive allopatric or parapatric divergence are hypothesized to drive

diversification within large species radiations (Rundell & Price, 2009; Glor, 2010; Losos, 2010), although the underlying processes remain poorly understood (Yoder *et al.*, 2010). Disentangling drivers of diversification requires systems with

well-resolved phylogenies and detailed ecological information (Glor, 2010; Losos, 2010). Oceanic islands offer powerful models for understanding mechanisms of diversification because an extensive body of theory provides testable predictions of how the geographical and ecological properties of islands influence endemic diversity (MacArthur & Wilson, 1967; Whittaker *et al.*, 2007, 2008; Gillespie & Baldwin, 2010). Moreover, these isolated landmasses have discrete geographical ranges, yielding tractable study systems within which to examine the evolution of novel ecological adaptations.

The formation of the Hawaiian Islands is expected to have shaped the diversification of its endemic lineages. The Hawaiian Islands are a hotspot archipelago, where islands form over an area of active volcanism and subsequently move north-west with the Pacific plate (Carson & Clague, 1995). This has generated a series of age-sorted islands, with the youngest located in the south-east and the oldest in the north-west of the chain (Fig. 1). Once shield building ceases, erosion and subsidence cause islands to shrink in both elevation and area, reducing the diversity of available habitat types (Carson & Clague, 1995). While island movement has been mostly constant over time, island formation has not, resulting in periods with many high islands and other times with few low islands (Price & Clague, 2002). This process has resulted in complex, temporally dynamic islands that comprise multiple volcanic peaks and replicated habitat structures.

Prior to the formation of Kauai, c. 5.1 million years ago (Ma), erosion reduced the older Northwestern Hawaiian

Islands to low-lying landmasses and atolls, probably eliminating many endemic lineages (Price & Clague, 2002). This resulted in a relatively recent biota derived almost entirely by *in situ* speciation with unprecedented levels of endemism: 99% for insects and snails, and 89% for plants (Miller & Eldredge, 1996; Wagner *et al.*, 1999). Once established, large species radiations were presented with novel ecological opportunities and a dynamic landscape, which may have led to initial bursts in net speciation rates (Gillespie & Baldwin, 2010; Glor, 2010; Rabosky & Glor, 2010). A null hypothesis for early colonists is a 'progression rule' biogeographical pattern in which lineage diversification occurred on progressively younger islands as they formed (see Wagner & Funk, 1995). This continual emergence of islands may have sustained high speciation rates as lineages diversified rapidly to fill them (Whittaker *et al.*, 2008). Nevertheless, as resource limits were reached through shrinking niche space (e.g. increased competition and island senescence: Silvertown, 2004; Whittaker *et al.*, 2008), diversity-dependent effects are expected to have caused declines in net speciation rates (Rabosky & Lovette, 2008a; Whittaker *et al.*, 2007, 2008; Rabosky & Glor, 2010). Alternative hypotheses predict that as species diversity increased so too did net diversification rates (Emerson & Kolm, 2005; see also Gruner *et al.*, 2008); however, these patterns remain to be tested for many endemic groups.

The Hawaiian Islands are home to several iconic adaptive radiations, including the Hawaiian *Drosophila* (O'Grady &

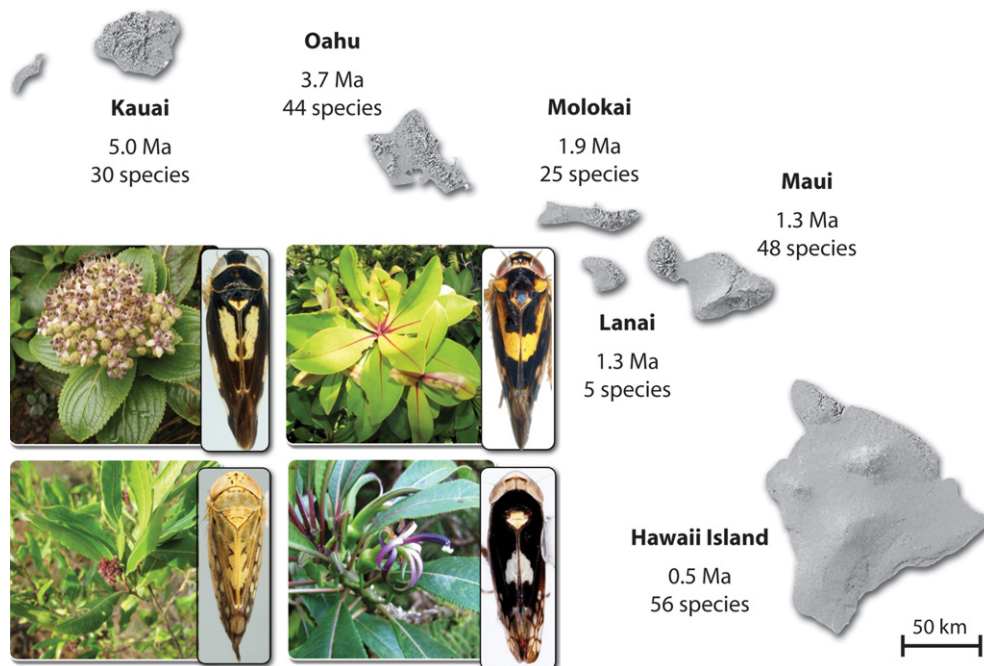


Figure 1 The current Hawaiian high islands. Island ages in millions of years (Ma) (Carson & Clague, 1995) and species sampling for native Hawaiian *Nesophrosyne* are given. The inset image shows common *Nesophrosyne* species and their host plant families (clockwise from the top left corner): *N. kanawao* & Hydrangeaceae: *Broussaisia*; '*N. sp. 125*' & Myrsinaceae: *Myrsine*; *N. nuenuue* & Sapindaceae: *Dodonaea*; and '*N. sp. 23*' & Campanulaceae: *Clermontia*.

Desalle, 2008), *Tetragnatha* (Gillespie, 2004), and silver-swords (Baldwin & Sanderson, 1998). However, large elements of the endemic biota are relatively poorly known, including sap-feeding insects in the suborder Auchenorrhyncha (Hemiptera), a clade that constitutes a major fraction of Hawaii's species diversity (Roderick & Percy, 2008). Phytophagous insects provide a unique opportunity to examine the role of ecological adaptation because they are obligately associated with the local flora and exhibit high levels of host-specificity (Price, 2008; Roderick & Percy, 2008). The intimate relationship between insects and their host plants can provide fundamental ecological opportunities for adaptive diversification to occur and may shape local insect diversity, geographical ranges and trait divergence (Cocroft *et al.*, 2008; Price, 2008).

This study focuses on *Nesophrosyne* leafhoppers (Hemiptera: Cicadellidae), the largest endemic clade of Hawaiian phytophagous insects, with over 190 known species. *Nesophrosyne* originated from the Western Pacific Rim, sharing a common ancestor with the widespread leafhopper genus *Orosius* (reviewed by Bennett & O'Grady, 2011, 2012). This group is characterized by high levels of local endemism, cryptic diversity, and host plant specificity (Bennett & O'Grady, 2012). Species exhibit dramatic morphological adaptations to the endemic Hawaiian flora, and diversification across the archipelago to fill all habitat types from coastal to sub-alpine regions (Bennett & O'Grady, 2012). We place *Nesophrosyne* in a time-calibrated comparative phylogenetic framework to ask how ecological and geological opportunities have influenced both the shape and tempo of species diversification in the group. Given the exceptional species diversity of *Nesophrosyne*, and its ecological ubiquity, we hypothesize that the genus established early on in the formation of the Hawaiian Islands. Thus, we expect that open ecological (e.g. host plants) and geographical opportunity offered by a young archipelago should have caused early, explosive species diversification. We further predict that these opportunities are limited, and as the Hawaiian Islands matured to their current state, strict limits on *Neosphrosyne* species diversity led to an eventual decline in diversification rate. Finally, given the reliance of *Nesophrosyne* on the native flora, we hypothesize that host plant associations (e.g. availability and arrival times) shaped species diversity and biogeographical patterns in the genus.

MATERIALS AND METHODS

Data

This study includes 191 *Nesophrosyne* species, represented by 198 individuals, and two *Orosius* species as outgroups. Initial molecular work screened over 360 individuals to determine species diversity in *Nesophrosyne* (Bennett & O'Grady, 2012). Our genetic data consist of a total of six genes (3520 base pairs) from the mitochondrial (cytochrome *c* oxidase subunits I and II, and 16S), nuclear (histone 3 and wingless), and

bacterial endosymbiont (16S) genomes. Species range and host plant data were taken from previous work on *Nesophrosyne* (Bennett & O'Grady, 2012).

Molecular dating

Time-calibrated phylogenetic analyses were undertaken using BEAST 1.6.2 on XSEDE in CIPRES (Drummond & Rambaut, 2007; Miller *et al.*, 2009). Markov chain Monte Carlo (MCMC) runs were implemented under a relaxed uncorrelated lognormal model, allowing for simultaneous estimation of phylogeny and divergence times, uncertainty in calibration specification, and rate heterogeneity among branches (Drummond *et al.*, 2006). Molecular data were fully partitioned by first, second, third codon positions, and ribosomal loci (Bennett & O'Grady, 2012).

Although no fossil calibrations are available for *Nesophrosyne* or the subfamily Deltocephalinae (Cicadellidae) (Zahniser & Dietrich, 2010), the Hawaiian Islands offer a well-tested suite of geological calibrations to infer the absolute ages of endemic groups (Carson & Clague, 1995; Fleischer *et al.*, 1998). Fleischer *et al.* (1998) proposed a set of criteria for determining geological calibrations, which were addressed objectively by reconstructing ancestral island associations on the Bayesian phylogeny of Bennett & O'Grady (2012) with maximum likelihood in MESQUITE 2.75 (Maddison & Maddison, 2011) (results not shown). Calibrations were selected for lineages exhibiting highly supported progression-rule patterns at highly supported nodes (Bayesian posterior probability ≥ 95). Nine calibration points were identified (see Appendix S1b in the Supporting Information), corresponding to Kauai, Maui and the island of Hawaii. An emphasis was placed on calibrating monophyletic radiations that progressed from Maui to Hawaii in order to reduce assumptions about the root divergence time for *Nesophrosyne*. The crown node for clade A was selected for an older calibration point since all basally diverging nodes reconstruct as Kauai (see Results), and it represents the most basally supported node other than the root. Normally distributed probabilistic priors were set with means centred on the biologically relevant age of each island (Carson & Clague, 1995; D.A. Clague, Monterey Bay Aquarium Research Institute, pers. comm.): island of Hawaii, mean 0.5 Ma [95% prior distribution (PD): 0.25–0.75 Ma]; Maui, mean 1.3 Ma (95% PD: 1.05–1.55 Ma); and Kauai, mean 5.1 Ma (95% PD: 4.17–5.82 Ma). The 95% intervals were set to allow for uncertainty in island colonization times, and were increased for Kauai, reflecting the long geological period during which it existed as the only high island (3.7–5.1 Ma; Carson & Clague, 1995). The prior distribution covers the margin of error for the K–Ar dating technique, which varies between 0.2 Ma for Kauai and 0.02 Ma for Hawaii Island (Clague & Dalrymple, 1987).

BEAST analyses were run four times independently for 1×10^9 generations sampled every 4000th iteration. Searches were initiated from a randomly generated starting tree under a Yule prior. Convergence and burn-in was assessed using

the estimated sample size (ESS) > 200 in TRACER 1.5 and cumulative posterior probability plots in AWTY (Rambaut & Drummond, 2007; Nylander *et al.*, 2008). Convergence diagnostics indicated that a 25% burn-in was adequate. A maximum clade credibility tree (MCCT) was summarized for 5000 post burn-in trees combined from each run.

Historical biogeographical and ancestral host plant reconstructions

The historical biogeography of *Nesophrosyne* was reconstructed using the dispersal–extinction–cladogenesis (DEC) maximum likelihood ancestral range reconstruction method in LAGRANGE 2 (Ree & Smith, 2008), which was chosen for its incorporation of uncertainty in ancestral range reconstructions, modelling of palaeogeographical information, and increased accuracy over other methods (Ree & Smith, 2008; Buerki *et al.*, 2011). Ancestral range areas were set as a seven-state model: Kauai (K), Oahu (O), Molokai (Mo), Lanai (L), Maui (M), Hawaii Island (H), and Australia (Aus, for *Orosius*). Reconstructions were conditioned on the MCCT tree. Four ancestral range models were run: (1) unconstrained (UM-U); (2) unconstrained two-area ancestral state (UM-2); (3) time-stratified unconstrained (TS-U); and (4) a time-stratified two-area ancestral state (TS-2). The unconstrained model allowed all possible ancestral range combinations for ancestral nodes, whereas the constrained model limited ancestral reconstructions to a two-state maximum (e.g. Kauai + Oahu). A two-state historical range model was implemented because the DEC method requires all terminal states to be considered in the inference of ancestral nodes, leading to inflated uncertainty (Ree & Sanmartín, 2009; Buerki *et al.*, 2011). The time-stratified models imposed a series of transition rate matrices that restricted lineages from colonizing islands prior to their formation. All models allowed equal transition rates between islands, reflecting the lack of information regarding inter-island dispersal rates. Model performance was evaluated based on geological plausibility (e.g. inferred ancestral ranges versus island formation times) and a statistically significant confidence window of > 2 log-likelihood units (Ree & Smith, 2008).

Ancestral host plant associations were reconstructed with maximum likelihood criteria in MESQUITE 2.75 (Maddison & Maddison, 2011). Reconstructions were performed on the MCCT, under a Markov *K*-state 1 (MK1) model. Host plant family categorical data were coded as 22 states, following Bennett & O’Grady (2012). The MK1 likelihood ancestral state reconstruction method was selected over other methods (e.g. DEC or Bayesian) for computational feasibility because the number of transition rates to be estimated for ancestral host plant associations is large.

Phylogenetic species clustering

Phylogenetic clustering of *Nesophrosyne* species was examined by estimating the standardized mean phylogenetic dis-

tance (MPD) and the mean nearest taxon distance (MNTD) with PICANTE 1.3 in R (R Development Core Team, 2009; Kembel *et al.*, 2010). The MPD measures the distance between taxon pairs through the root to the tips, generating an estimate of species clustering over the whole phylogeny. The MNTD measures clustering of terminal nodes. Negative standardized values (MPD.z and MNTD.z) are evidence for species clustering, and values ≥ 0 indicate phylogenetic evenness or overdispersion. The MPD and MNTD were estimated for the MCCT tree based on a six-state Hawaiian Island model, with outgroup and duplicate species removed. Statistical significance was assessed by generating a null distribution of 1000 phylogenies with tips randomly reshuffled.

Diversification rate analyses

Diversification dynamics in *Nesophrosyne* were inferred using R statistical packages APE and LASER (Paradis *et al.*, 2004; Rabosky, 2006; R Development Core Team, 2009) with outgroup and duplicate taxa removed. A lineage-through-time (LTT) plot was constructed from the MCCT with 95% confidence intervals derived from 1000 trees drawn from the Bayesian posterior distribution. The constant rate (CR) test of Pybus & Harvey (2000) was used to investigate shifts in net diversification rates. A γ -value less than -1.645 rejects a constant rate hypothesis, indicating a rate slowdown (Pybus & Harvey, 2000). Incomplete taxon sampling (e.g. incomplete collections or extinction among lineages) was addressed using Monte Carlo simulations of the CR test (MCCR) conducted under stepwise increases of 5% missing taxa until statistical significance decayed (P -value > 0.01). Null distributions were simulated for 5000 phylogenies under an empirically determined rate-constant, pure-birth process for some number of species, with terminal nodes trimmed to the desired percentage of missing taxa. Phylogenetic uncertainty was assessed by estimating the maximum γ -value for 1000 trees drawn from the Bayesian posterior distribution.

Temporal shifts in net diversification rates were estimated using birth–death likelihood (BDL) methods in LASER (Rabosky, 2006). BDL methods offer the opportunity to test a priori hypotheses of diversification rates shifts and provide superior power over the γ -statistic to detect shifts in diversification when background extinction is non-zero (Rabosky, 2006). The best-fitting models were determined by estimating the likelihood of rate constant (RC) and rate variable (RV) models, and statistically evaluated with the Akaike information criterion ($\Delta\text{AIC}_{\text{RC}} = \text{AIC}_{\text{RC}} - \text{AIC}_{\text{RV}}$). A positive $\Delta\text{AIC}_{\text{RC}}$ indicates support for a rate variable model. Two rate-constant models and four rate-variable models were tested: (1) pure birth (Yule); (2) constant-rate birth–death (B–D); (3) logistic diversity-dependent (DDL); (4) exponential diversity-dependent (DDX); (5) a Yule-2-rate model; and (6) a Yule-3-rate model. Statistical significance was assessed by simulating a null distribution of 5000 trees under an empirical rate-constant pure-birth model for complete and

incomplete sampling (50% and the 80% CR test breaking point). Phylogenetic uncertainty was addressed by calculating the $\Delta\text{AIC}_{\text{RC}}$ for 1000 posterior Bayesian trees. The null hypothesis of rate constancy was rejected if the observed $\Delta\text{AIC}_{\text{RC}}$ for the MCCT fell outside the 95% interval of the null distributions ($P < 0.01$).

RESULTS

Molecular dating

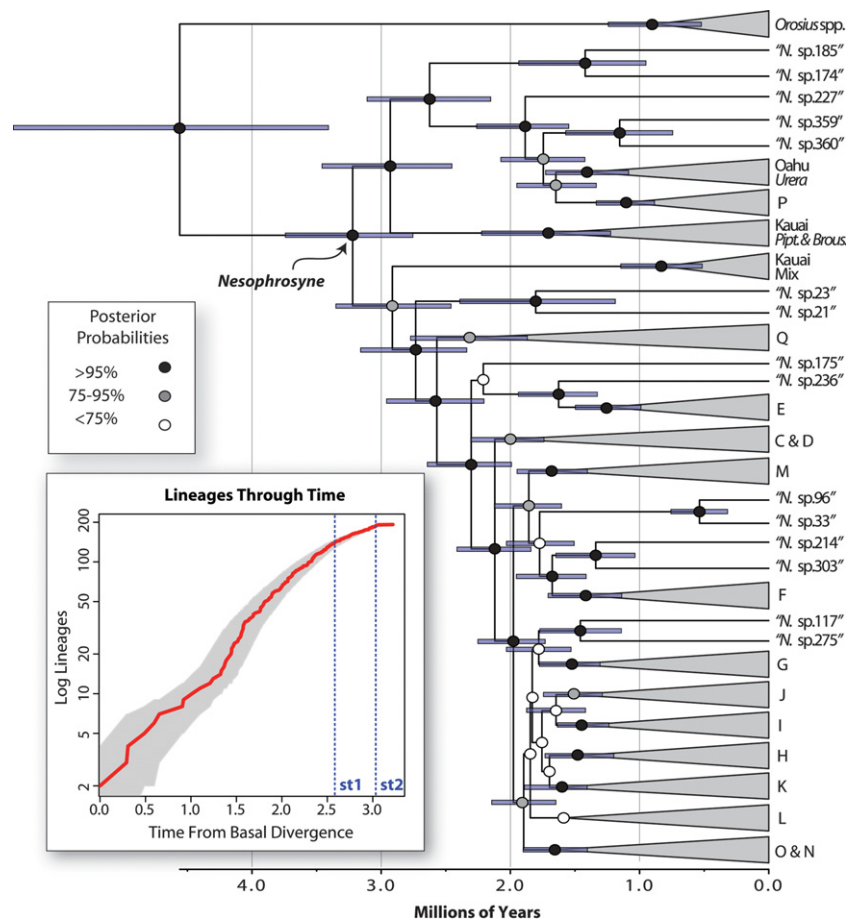
The Bayesian MCCT (Fig. 2) has a similar topology to that of Bennett & O'Grady (2012). For clarity, clades are given letter names (A–Q). Posterior support for many relationships is moderate to high [posterior probability (PP) = 0.75–1], although several mid-level nodes received low or no support (PP < 0.5). The split between *Nesophrosyne* and *Orosius* is reconstructed as 4.5 Ma [95% highest posterior density (HPD): 3.41–5.84 Ma], with the root divergence in *Nesophrosyne* at 3.2 Ma (95% HPD: 2.75–3.74 Ma). *Nesophrosyne* is composed of two clades, A and B, with basal divergence within them at 2.93 Ma (95% HPD: 2.45–3.46 Ma) and 2.91 Ma (95% HPD: 2.46–3.35 Ma), respectively. Statistics for MCMC runs and complete node ages are summarized in Appendix S1.

Historical biogeography and ancestral host plant reconstructions

Performance of the biogeographical models varied by more than 2 log-likelihood (lnL) units: UM-U = −358.8; UM-2 = −367.9; TS-U = −364.8; TS-2 = −375.3. The unconstrained UM-U and TS-U models yielded significantly lower likelihood scores, but also inferred ancestral area reconstructions at basal nodes that are geologically implausible. For example, both models inferred ancestral island ranges at the root and at the base of clade A that included the youngest islands, Maui and Hawaii (results not shown). However, the inferred ages were between 2.5 and 3.2 Ma – far older than the emergence times of Maui and Hawaii at 1.3 and 0.5 Ma, respectively. Therefore, the TS-U and UM-U models were rejected.

The UM-2 and TS-2 models gave similar results, although UM-2 (Fig. 3) had a significantly better likelihood score than TS-2 (> 2 lnL units). Both the UM-2 and TS-2 models reconstructed Kauai as the ancestral area for the root node of *Nesophrosyne* with strong support [probability (Prob) = 0.77–0.81]. The base of clade A was reconstructed as Kauai (Prob = 0.73–0.76). The basally diverging nodes for clade B gave a highly supported Kauai origin, with a mid-depth transition to Oahu (Prob = 0.68–0.78, PP =

Figure 2 Time-calibrated maximum clade credibility tree (MCCT) for native Hawaiian *Nesophrosyne*. Taxonomic and genetic sampling included 201 taxa, representing 191 species, and two *Orosius* outgroup species for six genes (c. 3.5 kb). Bayesian analyses were conducted under a relaxed lognormal clock model in BEAST (Drummond *et al.*, 2006). Summary statistics for each run are presented in Appendix S1a. The inset legend explains posterior node support. Clades have been collapsed for clarity (see Appendix S1b). The grey bars show the 95% highest posterior density (HPD) for age estimates. The inset graph shows the log lineages-through-time plot for the MCCT. The line represents the MCCT, with the grey polygon illustrating the 95% posterior distribution for 1000 Bayesian trees. The dashed lines illustrate the transition points for diversification rate declines (*st1* and *st2*) for the Yule-3-rate model determined with LASER (Rabosky, 2006).



0.96–0.98). More derived clades exhibited complex biogeographical patterns (see Appendix S2) that include progression of lineages from younger to older islands (clades C, G, I, K, M, N and O), island skipping (C, F and N), and back colonization (D, E, J and N). Historical biogeographical reconstructions also reveal extensive intra-island diversification in all clades (see Appendix S2 for full reconstructions).

Ancestral host plant reconstructions for all major clades are shown in Fig. 3. The ancestral state for the root node is equivocally split between Urticaceae (Prob = 0.40) and Rubiaceae (Prob = 0.38). The base of clade A is reconstructed as Urticaceae (Prob = 0.98), with all subsequently diverging nodes receiving high support for an Urticaceae association (Prob > 0.98). The ancestral host association for the base of clade B is Rubiaceae, with subsequently diverging nodes receiving progressively higher support for a Rubiaceae association (Prob = 0.62–0.99). Within clade B, host-specific clades emerge from highly supported associations with Rubiaceae. See Appendix S3 for complete reconstructions.

Phylogenetic clustering

The MPD indicated that Kauai endemic species were significantly clustered (Table 1: MPD.Z = -6.46, $P < 0.001$). Clustering on all other islands was non-significant (MPD.Z = -1.21–2.09, $P = 0.12–0.99$). MNTD demonstrated highly significant clustering on all islands (MNTD.z = -5.15 to -7.43, $P = 0.001$) except Molokai and Lanai (MNTD.z = -1.25 & 0.34, $P = 0.10$ & 0.64).

Diversification rate analyses

The lineage-through-time plot for the MCCT is shown in Fig. 2. The CR test γ -statistic (Table 2) is negative ($\gamma = -8.48$) and highly significant ($P < 0.001$) when complete sampling is assumed. This result is robust to MCCRS simulations of incomplete sampling with statistical significance beginning to decay at 80% and 85% missing taxa ($\gamma = -7.61$ & -8.53 , $P = 0.002$ & 0.06), and phylogenetic

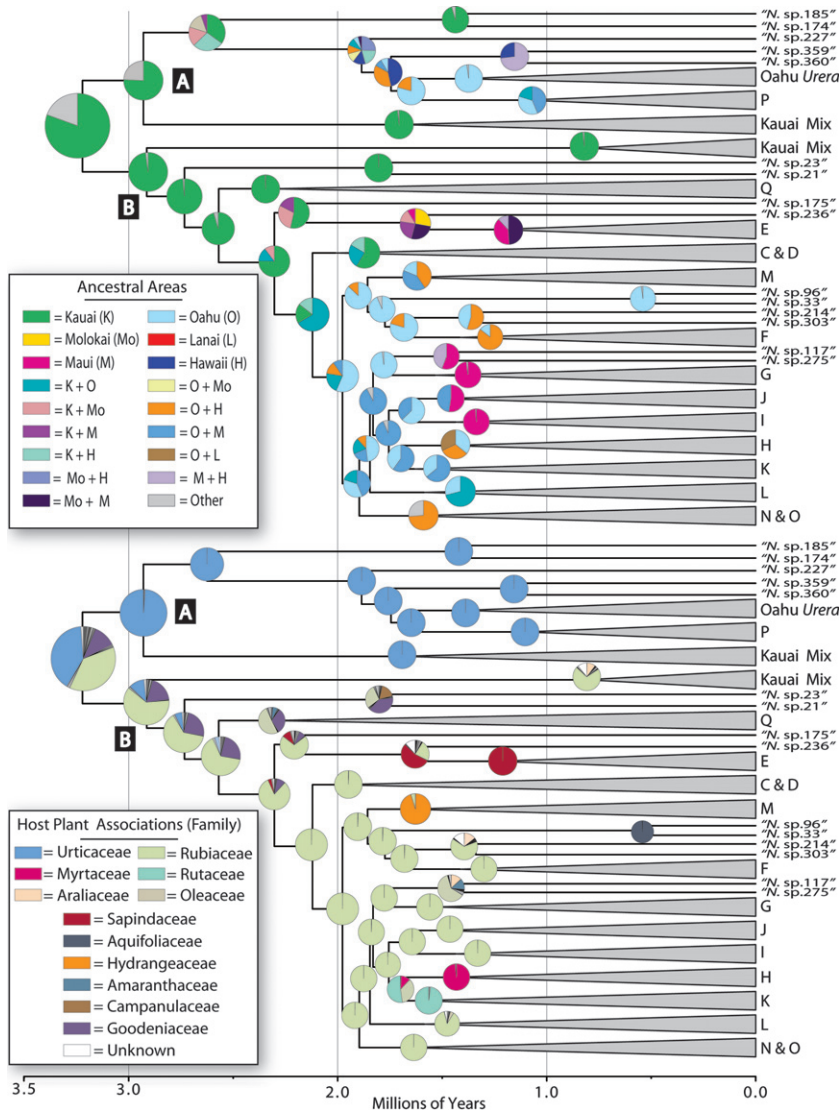


Figure 3 Maximum likelihood reconstructions for ancestral island associations and host plant associations for 191 endemic Hawaiian *Nesophrosyne* species. Outgroups have been trimmed. The top tree shows reconstruction of ancestral island associations under the UM-2 model using LAGRANGE (Ree & Smith, 2008). The bottom tree shows host plant ancestral state reconstructions using MESQUITE (Maddison & Maddison, 2011). Pie charts illustrate the relative probability for each state. Major clades have been collapsed to show overall patterns of ancestral states (see Appendices S2 & S3). Letters in black boxes refer to clade names.

uncertainty with the maximum γ -value (-7.38) for 1000 posterior trees remaining statistically significant until 75% missing taxa ($P > 0.01$).

The $\Delta\text{AIC}_{\text{RC}}$ test statistic (Table 2) for the MCCT rejected constant rate diversification models ($P < 0.001$), with the Yule-3-rate model receiving the lowest AIC score over all multi-rate models. The Yule-3-rate model approximated two transition points occurring 0.63 Ma (*st1*) and 0.17 Ma (*st2*), with a stepwise decline in net speciation rates ($r1 = 1.51$, $r2 = 0.62$ and $r3 = 0.03$: see Fig. 2). Results are robust to phylogenetic uncertainty and incomplete sampling, with the

$\Delta\text{AIC}_{\text{RC}}$ for the posterior distribution falling outside the simulated null distributions for complete and incomplete sampling.

DISCUSSION

Age and establishment of *Nesophrosyne*

Nesophrosyne split from its sister genus, *Orosius*, 4.5 Ma (95% HPD: 3.41–5.84 Ma), with a subsequent basal divergence on Kauai, 3.2 Ma (95% HPD: 2.75–3.74 Ma). Previous taxonomic and phylogenetic evidence suggests *Nesophrosyne* colonized the Hawaiian Islands from the Western Pacific rim (reviewed by Bennett & O'Grady, 2011, 2012). Prior to the emergence of Kauai, *c.* 5.1 Ma, geologically driven extinction stripped the islands of most lineages and complex habitat structures suitable to support these lineages or newly colonizing species (Price & Clague, 2002). The regional assembly of the endemic biota on the current Hawaiian islands is derived from colonists arriving during two distinct time periods: (1) prior to *c.* 5–7 Ma when the ancient high islands were still extant but had subsided to the point where little or no wet forest was present; and (2) since *c.* 5 Ma when the current high islands formed with a dynamic diversity of wet and mesic forest habitats (Price & Clague, 2002). Until the formation of Oahu (3.7–3.2 Ma), Kauai was the highest and only ecologically mature sub-aerial landmass, and the most likely point of origin for colonizing lineages (Price & Clague, 2002). While several groups of endemic Hawaiian taxa pre-date Kauai (e.g. Givnish *et al.*, 2009; Rubinoff & Schmitz, 2010), the bulk of the endemic biota has diversified since its emergence (Price & Clague, 2002).

Once *Nesophrosyne* became established in the Hawaiian Islands, competition between other insect herbivores and the periodic arrival of new plant lineages may have shaped

Table 1 Phylogenetic clustering statistics for 191 native Hawaiian *Nesophrosyne* species, according to species' island of endemism using PICANTE (Kembel *et al.*, 2010).

	ntaxa	mpd.obs.z	P-value
Mean phylogenetic distance (MPD)			
Hawaii	56	2.09	0.99
Kauai	30	-6.46	0.001
Lanai	5	-0.45	0.33
Maui	48	-1.21	0.12
Molokai	25	0.24	0.60
Oahu	44	-0.09	0.47
	ntaxa	mntd.obs.z	P-value
Mean nearest taxon distance (MNTD)			
Hawaii	56	-6.63	0.001
Kauai	30	-6.03	0.001
Lanai	5	0.34	0.64
Maui	48	-5.15	0.001
Molokai	25	-1.25	0.10
Oahu	44	-7.43	0.001

ntaxa, number of species per island; mpd and mntd.obs.z, standardized effect size.

Table 2 Diversification rate results for the constant rate test (Pybus & Harvey, 2000) and birth–death likelihood model fitting (Rabosky, 2006) for 191 endemic Hawaiian *Nesophrosyne* species. Analyses were performed using the Bayesian maximum clade credibility tree (MCCT) and 1000 posterior Bayesian trees. Statistical significance was assessed by simulating a null distribution of 5000 trees at intervals of 5% missing taxa.

Pure birth	Birth–death	DDL	DDX	Yule-2-rate	Yule-3-rate	Best model: $\Delta\text{AIC}_{\text{RC}}$ P-values	γ -statistic MCCR tests (P-value)
LH = 609.09	LH = 609.09	LH = 647.71	LH = 628.10	LH = 640.21	LH = 653.50	Yule-3-rate	<i>Nesophrosyne</i> MCCT:
$\Delta\text{AIC} = 80.82$	$\Delta\text{AIC} = 82.82$	$\Delta\text{AIC} = 5.57$	$\Delta\text{AIC} = 44.81$	$\Delta\text{AIC} = 22.58$	$\Delta\text{AIC} = 0$	80.82	-8.48
$r = 0.9365467$	$r = 0.94$	$r1 = 2.46$	$r1 = 7.08$	$r1 = 1.51$	$r1 = 1.51$	Complete	MCCR 80% missing
	$a = 0$	$k = 210.13$	$x = 0.45$	$r2 = 0.44$	$r2 = 0.62$	sampling:	taxa:
				$st1 = 0.63$	$r3 = 0.03$	$P < 0.001$	-7.61 ($P < 0.02$)
					$st1 = 0.63$	50% missing taxa:	MCCR 85% missing
					$st2 = 0.17$	$P < 0.001$	taxa:
						80% missing taxa:	-8.53 ($P = 0.06$)
						$P < 0.001$	

DDL, logistic diversity-dependent model; DDX, exponential diversity-dependent model.

Parameter abbreviations: LH, log likelihood; ΔAIC , difference in Akaike information criterion score; r , speciation rate for multi-rate models; a , extinction rate; k , logistic; x , exponential; st , transition for rates shifts in multi-rate Yule models; MCCR, Monte Carlo constant rate test.

diversification and host plant specialization patterns in the group. The genus is the largest leafhopper radiation on Hawaii, with at least 190 species. The next largest cicadellid genus, *Balclutha* (also a member of the Deltocephalinae subfamily), comprises a mere 10 endemic species largely restricted to grasslands (Knight, 1987). Highly competitive or early establishing lineages are expected to form large monophyletic groups, like *Nesophrosyne*, by pre-emptively excluding other lineages from establishing in Hawaii or from occupying certain niches (Silvertown, 2004). The overall disharmony in leafhopper lineages, which is dominated by a single genus and subfamily, and the limited species diversity and habitat use in *Balclutha* may be due to direct competition that prevented the broad establishment of rival groups. This process can influence both the local and regional assembly and phylogenetic structure of endemic biota (Silvertown, 2004; reviewed by Emerson & Gillespie, 2008). At the local level, pre-emptive exclusion appears to have further shaped diversity within *Nesophrosyne* as well, which led to the formation of large clades of host plant specialists exclusively associated with elements of the endemic flora (e.g. clade A, Urticaceae; M, Hydrangeaceae: *Broussaisia*; and E, Sapindaceae: *Dodonaea*). Presumably, lineage-specific competitive advantages prevented sister lineages from overlapping in host plant associations.

Competition between distantly related endemic sap-feeding insects might have excluded rival radiations from specializing on certain host plants. Ecological evidence suggests that this process was important in shaping *Nesophrosyne* diversity, which is generally absent from the oldest known primary plant lineages, the silversword alliance (Asteraceae) and the lobelioids (Campanulaceae), and other common elements of the endemic flora, including *Acacia koa* (Fabaceae) and *Cyrtandra* spp. (Gesneriaceae) (Bennett & O'Grady, 2012). No known *Nesophrosyne* species are associated with Asteraceae, and specialization on lobelioids is rare (Bennett & O'Grady, 2012), although both are dominant hosts for the *Nesosydne* planthoppers (Delphacidae), which share the same basic niche (phloem feeding, oviposition and nymphal development: Roderick & Percy, 2008). The species-rich radiations, the lobelioids and silverswords, are estimated to have established prior to *Nesophrosyne* at 13.6 Ma and 5.2 Ma, respectively (Baldwin & Sanderson, 1998; Givnish *et al.*, 2009). This suggests that these hosts were available to earlier colonizing lineages. While the age of Hawaiian *Nesosydne* is unknown, a compelling scenario emerges in which planthoppers arrived in the Hawaiian Islands prior to *Nesophrosyne* and pre-emptively excluded them from these host plants.

Historical biogeography

Historical biogeographical patterns in *Nesophrosyne* are complicated, indicating the highly dispersive nature of these insects. While some clades exhibit a general progression from older to younger islands (clades G, I, K and M), others show complex patterns: back dispersals to older islands (E, M and

P), island skipping (C and N), and uncertainty in historical inference (A). The historical biogeographical patterns in *Nesophrosyne* are largely governed by a complex interplay between host plant associations (discussed below) and island geography. Complex biogeographical patterns are characteristic of endemic radiations of other volant insect groups, including the *Hylaeus* bees and *Thyrocopa* moths (Magnacca & Danforth, 2006; Madeiros & Gillespie, 2011).

Lack of significant phylogenetic clustering in MPD analyses for most islands corroborates the dispersive nature of *Nesophrosyne*. Kauai does show significant clustering; this result may be due to the origin and early restriction of *Nesophrosyne* to Kauai, the greater isolation of Kauai relative to the other islands (128 km versus a maximum of 48 km between any other two high islands), or because Kauai is the oldest island and *in situ* speciation is expected to largely account for local species diversity there (Emerson & Gillespie, 2008). MNTD analyses, in contrast, show significant clustering of species at terminal tips, perhaps owing to intra-island diversification or shared dispersal routes between islands (i.e. colonization from older to younger islands). While communities on young islands are expected to be drawn from neighbouring source pools (Emerson & Gillespie, 2008), biogeographical reconstructions in *Nesophrosyne* suggest that intra-island diversification is more prevalent in the diversification of this genus. For example, most derived clades (see Appendix S3) represent monophyletic groups that diversified on a single island, with one locally endemic allopatric sibling species per volcano (Bennett & O'Grady, 2011, 2012). For these clades, the reconstructed origin for the crown node is consistent with the geological location of species at terminal tips (e.g. clades M, N and P).

Historical host plant associations

The emergent diversification pattern in *Nesophrosyne* is due, at least in part, to novel ecological opportunity related to endemic host plant diversity. *Nesophrosyne*'s extant species diversity is organized by host plant associations in over 21 families (Bennett & O'Grady, 2012). The colonizing ancestor of *Nesophrosyne* is equivocally reconstructed as associated with two plant families, Urticaceae and Rubiaceae, which may reflect a polyphagous ancestor. Subsequent basal divergences on Kauai are associated with *in situ* ecological specialization that organized the genus into two parallel clades: clade A, which is almost entirely associated with Urticaceae (only *Nesophrosyne heopoko* is found on Hydrangeaceae); and clade B, a complex of host-specific clades using over 20 plant families derived from an ancestral association with Rubiaceae. Genera in both Urticaceae (*Urera* and *Pipturus*) and Rubiaceae (*Coprosma*, *Psychotria* and *Kadua*) are widely used *Nesophrosyne* hosts and comprise a prominent element of Hawaii's mesic and wet-forest ecosystems (Wagner *et al.*, 1999). The evolutionarily restricted host plant association of clade A relative to clade B is striking. It is plausible that adaptation to Rubiaceae equipped *Nesophrosyne* species with

an adaptive flexibility to exploit other endemic plant lineages.

Host plant transitions in *Nesophrosyne* permitted colonization and allopatric speciation between islands and volcanoes within the Hawaiian Archipelago. A key feature of species diversity in the genus is the parallel diversification of *Nesophrosyne* clades restricted to different endemic host plant groups across each of the islands. The periodic arrival of host plants throughout the formation of the Hawaiian Islands provided a source of temporally dynamic ecological opportunity for range expansion in *Nesophrosyne*. For example, nested clades exhibit complicated biogeographical patterns associated with transitions to possibly more recently established Hawaiian plant taxa, including *Dodonaea* (Sapindaceae, clade E), *Myoporum* (Scrophulariaceae, D), and *Pipturus* (Urticaceae, P). Following transitions to these hosts, lineages dispersed to other islands in a reverse progression or stochastic fashion. The arrival time has been estimated only for *Dodonaea viscosa*, which represents a young lineage that established in the Hawaiian Islands within the last 0.5–1.2 Ma (Harrington & Gadek, 2009). The emergence of *Nesophrosyne* species in clade E is associated with specialization on *D. viscosa* and basal divergence on Maui 1.2 Ma (95% HPD: 0.99–1.50 Ma) with two parallel dispersals to Oahu and Hawaii Island.

Diversification dynamics of *Nesophrosyne*

Theoretical work in island biogeography and adaptive radiation predict that novel ecological opportunities will lead to early bursts in net speciation rate, but diversity-dependent slowdowns will follow as niches fill and islands senesce (Phillimore & Price, 2008; Rabosky & Lovette, 2008b; Whittaker *et al.*, 2007, 2008). *Nesophrosyne* exhibited an initially high net species diversification rate that lasted from 3.2 to 0.67 Ma, with a stepwise decline. The magnitude of the initial speciation rate ($r_1 = 1.51$) was more than double the following diversification rate ($r_2 = 0.62$) and 50 times higher than the final rate ($r_3 = 0.03$). The rapid net diversification rate is associated with host plant transitions on Kauai, and coincides with geological expansion of the Hawaiian Islands – the rapid growth of the multi-volcano islands Oahu and Maui Nui (3.7–0.75 Ma: Carson & Clague, 1995). This suggests that *Nesophrosyne* established during a period of open ecological opportunity that was expanding with the archipelago and maturing into complex replicated habitats across Hawaii's volcanic ranges.

The net speciation rate decline in *Nesophrosyne* is hypothesized as a diversity-dependent response to the exhaustion of ecological opportunity and geophysical area (Phillimore & Price, 2008; Rabosky & Lovette, 2008b). Transition times between rate declines are tightly correlated with the formation of the youngest islands: the first transition occurred approximately 0.67 Ma, which is congruent with an island building pause after the formation of Maui Nui (0.8 Ma) and prior to Hawaii (0.5 Ma); and the second transition

occurred 0.16 Ma, shortly after the formation of Mauna Loa (0.2 Ma), which marks the end point for the formation of the current high islands (Carson & Clague, 1995). The geologically correlated, precipitous rate decline indicates that ecological opportunity, related to host plant availability, may have been greatly reduced by the time Maui fully formed. *Nesophrosyne* diversification on the youngest islands is largely driven by habitat replication and subsequent allopatric speciation, which is supported by the fact that the volcanoes of Maui and Hawaii comprise locally endemic sibling species restricted to the same host lineages (Bennett & O'Grady, 2012). These results suggest that *Nesophrosyne* diversity may have reached equilibrium dynamics controlled by island area (MacArthur & Wilson, 1967; Gillespie & Baldwin, 2010).

Taken together, these results largely confirm the predictions of the general dynamic model of island biogeography (and its precursor, the island immaturity–speciation pulse model; Whittaker *et al.*, 2007, 2008). This model predicts: (1) that early geographical and ecological opportunities drive initially high speciation rates as new islands emerge; (2) that species diversification should transition to non-adaptive mechanisms as islands mature into complex landscapes; and (3) that finite island resources impose limits on species diversity (Whittaker *et al.*, 2007, 2008). In *Nesophrosyne*, these predictions are borne out. The observed early rapid diversification was associated with adaptation to novel host plants and the rapid growth of a young Hawaiian Archipelago. This was followed by a period of non-adaptive diversification as *Nesophrosyne* spread across Hawaii's volcanoes and geological features. Finally, diversification rates in the group declined as species exhausted available ecological and geographical opportunities. Moreover, this result contradicts the expectation that increasing species diversity should continue to sustain or increase diversification rates (Emerson & Kolm, 2005; Gruner *et al.*, 2008). While increasing competition may have contributed to the initial burst in *Nesophrosyne* diversity, it does not appear to have sustained species diversification (Emerson & Kolm, 2005). Instead, the diversification rate in *Nesophrosyne* declined with increasing species diversity.

Several caveats apply to diversification rate analyses regarding potential sources of error, including taxonomically biased sampling, ephemeral species diversity, rate heterogeneity, and unknown clade age and size (Phillimore & Price, 2008; Cusimano & Renner, 2010; Rosenblum *et al.*, 2012). These factors are difficult to assess for large insect radiations, which can be challenging to circumscribe taxonomically and ecologically. Our sampling targeted all major habitats across each volcano, all native plants, and included the known cryptic diversity in *Nesophrosyne*. Despite the fact that we include 48 of the 72 named *Nesophrosyne*, many of the existing species descriptions consist of only a few sentences that describe highly variable external characters, and cannot be used to accurately identify the extant diversity (reviewed by Bennett & O'Grady, 2011). Thus, our species level sampling is more robust than taxonomic estimates suggest, and probably

includes most of the previously observed diversity; however, names cannot be assigned until these species identities are clarified and formally redescribed using current taxonomic conventions. Finally, structured sampling may violate assumptions of random sampling, leading to the overestimation of rate variability and decline in *Nesophrosyne* net diversification rate (Cusimano & Renner, 2010). However, our sampling should buffer against over-representation of basally diverging lineages, and help ameliorate potential issues arising from typically undetected young species diversity (Rosenblum *et al.*, 2012).

Extinction remains an important consideration when inferring diversification rates (Ricklefs, 2010). Large extinction events are known to have occurred periodically in the Hawaiian Islands because of geological processes (Price & Clague, 2002) and anthropogenic causes (Cuddihy & Stone, 1990). At least 9% of the native flora is considered extinct, and more than 50% is at risk (Sakai *et al.*, 2002). The islands of Maui Nui, which host 40% of the extant *Nesophrosyne* species diversity, have the highest numbers of extinct taxa (Sakai *et al.*, 2002). The most heavily affected habitats include low elevation shrublands, mesic forests, and montane and wet-montane forests (Cuddihy & Stone, 1990; Sakai *et al.*, 2002) – all areas of high *Nesophrosyne* diversity. These extinction patterns may have significant lineage-specific impacts, biasing diversification rate analyses (Purvis, 2008); however, our results indicate that extinction may not have been a dominant force in shaping *Nesophrosyne* diversity. Historically high rates of extinction should erase evidence of early lineage accumulation, leading to an abundance of young lineages and an apparent increase in diversification through time (Pybus & Harvey, 2000). The opposite pattern is observed in *Nesophrosyne*. Regardless, diversification results for Hawaiian radiations should be interpreted cautiously because extinction is unable to be accurately estimated and its effects will forever remain elusive.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Summary statistics (a) and full phylogeny (b) for time-calibrated results of Bayesian molecular dating analyses for native Hawaiian *Nesophrosyne*.

Appendix S2 Maximum likelihood reconstructions of ancestral island associations for endemic Hawaiian *Nesophrosyne* species.

Appendix S3 Complete maximum likelihood ancestral host plant reconstructions for native Hawaiian *Nesophrosyne*.

BIOSKETCHES

Gordon M. Bennett is a PhD graduate from UC, Berkeley and is currently a postdoc at Yale. His research interests include the evolution, historical biogeography and adaptive radiation of endemic Hawaiian herbivorous insects. He is also interested in the evolution of host plant–insect interactions and bacterial endosymbioses.

Patrick M. O’Grady is an associate professor at UC, Berkeley. His research focuses on the ecology, evolution, biogeography and genomics of Hawaiian insects. He is also interested in understanding the role of plant–insect–microbe interactions in adaptive radiations, particularly within the large radiation of endemic Drosophilidae.

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