

COLONIZATION HISTORY AND POPULATION GENETICS OF THE COLOR-POLYMORPHIC HAWAIIAN HAPPY-FACE SPIDER *THERIDION GRALLATOR* (ARANEAE, THERIDIIDAE)

Peter J. P. Croucher,^{1,2} Geoff S. Oxford,³ Athena Lam,¹ Neesha Mody,¹ and Rosemary G. Gillespie¹

¹Department of Environmental Science, Policy, and Management, 130 Mulford Hall, University of California, Berkeley, California 94720–3114

²E-mail: croucher@berkeley.edu

³Department of Biology, University of York, Wentworth Way, Heslington, York YO10 5DD, United Kingdom

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Past geological and climatological processes shape extant biodiversity. In the Hawaiian Islands, these processes have provided the physical environment for a number of extensive adaptive radiations. Yet, single species that occur throughout the islands provide some of the best cases for understanding how species respond to the shifting dynamics of the islands in the context of colonization history and associated demographic and adaptive shifts. Here, we focus on the Hawaiian happy-face spider, a single color-polymorphic species, and use mitochondrial and nuclear allozyme markers to examine (1) how the mosaic formation of the landscape has dictated population structure, and (2) how cycles of expansion and contraction of the habitat matrix have been associated with demographic shifts, including a “quantum shift” in the genetic basis of the color polymorphism. The results show a marked structure among populations consistent with the age progression of the islands. The finding of low genetic diversity at the youngest site coupled with the very high diversity of haplotypes on the slightly older substrates that are highly dissected by recent volcanism suggests that the mosaic structure of the landscape may play an important role in allowing differentiation of the adaptive color polymorphism.

KEY WORDS: Adaptive shifts, founder effects, phylogeography.

The Hawaiian archipelago was formed de novo by volcanic activity, with the islands generated in a chronological sequence as the Pacific plate moved over a “hot spot” in the earth’s crust (Fig. 1). This temporal arrangement, coupled with its isolation (3200 km from the nearest continent), and the diversity of geological processes and microclimates within each island, renders the archipelago an ideal location for the study of evolution. Isolation has allowed the development of some of the most dramatic known examples of adaptive evolution. Although many of these are involved with species proliferation (Cowie and Holland 2008; Rundell and Price 2009), there are some cases in which pop-

ulation differentiation has occurred without speciation (Givnish 1997), for example, molecular diversification of the land snail *Succinea caduca* within and among six of the Hawaiian Islands (Holland and Cowie 2007), and complex population structuring in the damselflies *Megalagrion xanthomelas* and *M. pacificum* (Jordan et al. 2005). These latter cases provide a unique opportunity to examine the role of the dynamic landscape in fostering genetic differentiation and adaptive stasis (Carson et al. 1990).

The biogeographic pattern that predominates in Hawaiian taxa, for both species and populations, is a step-like progression down the island chain from older to younger islands (Wagner and



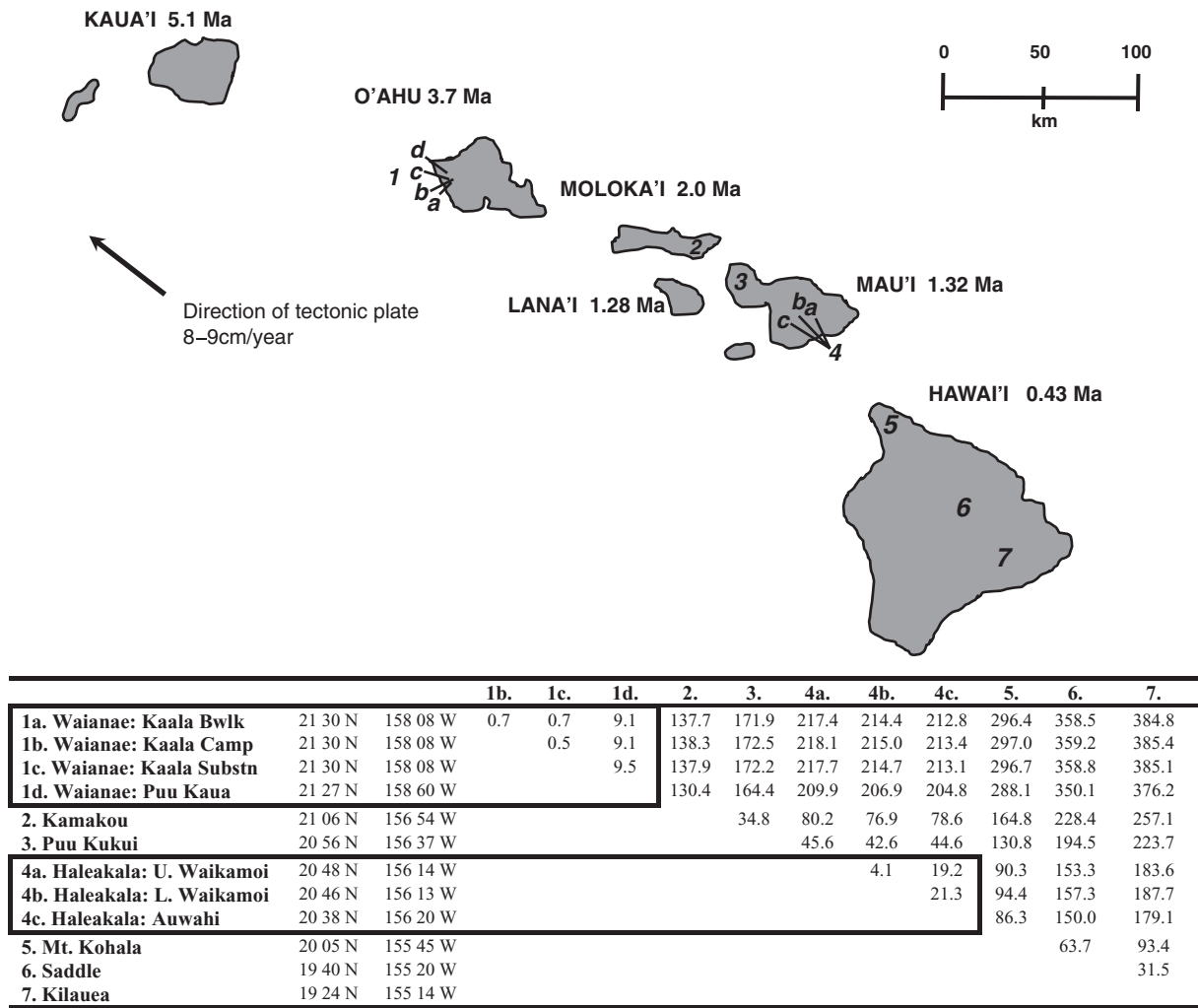


Figure 1. Map of the Hawaiian archipelago indicating the *Theridion grillator* sample sites numbered according to the volcano on which they were collected. Island ages are given according to Clague (1996): (1) Waianae, Oahu (Mt. Kaala Boardwalk [1a]—a trail through the Mt. Kaala bog-forest, Camp [1b]—a forest-surrounded clearing off the Mt. Kaala access road, Substation [1c]—forest adjacent to a small electricity generator station belonging to the FAA—all elevation 1200 m, and adjacent Puu Kaua [1d], elevation 950 m); (2) Kamakou, Molokai, elevation 1110 m; (3) Puu Kukui, West Maui – Maui Land and Pineapple Company’s Puu Kukui Watershed Preserve, elevation 1700 m; (4) Haleakala, East Maui (Upper Waikamoi, elevation 1700 m [4a], Lower Waikamoi [4b], elevation 1360 m, Auwahi Forest [4c]—an area of remnant dry forest (Wagner et al. 1999), elevation 1200 m); (5) Mt. Kohala, Hawaii – Kahua Ranch, elevation 1152m; (6) Mauna Kea/Mauna Loa Saddle, Hawaii, elevation 1600m; (7) Kilauea, Hawaii-Thurston Lava Tube, Hawaii Volcanoes National Park, elevation 1190 m. Samples from (6), the Saddle, came from two Kipuka (“18” and “19”)—patches of rainforest isolated by lava flows (Vandergast et al. 2004)—numbered according to their proximity to the Saddle Road mile markers. Sites 1a, 1b, 1c, 1d, 2, 4a, and 4b were all on preserves of The Nature Conservancy of Hawaii. The table below the map shows precise locations of collecting sites, with the distance between each (km) with the site 1 Waianae and site 4 Haleakala subsites boxed.

Funk 1995) often with repeated bouts of diversification within islands and with limited gene flow between islands (Roderick and Gillespie 1998). However, the progression is complicated by historical connections between some of the islands. In particular, the islands of Maui, Molokai, Lanai, and Kahoolawe—often referred to as Greater Maui (Maui Nui)—were formed (1.2–2.2 Ma) as a single landmass and remained as such until subsidence started causing separation of the islands (0.6 Ma), with intermittent connection since that time during low sea stands (Price and

Elliot-Fisk 2004). Likewise, the repeated episodes of extinction and recolonization resulting from active volcanism, currently evidenced on Hawaii Island, were presumably also operating on the older islands during their formation.

Carson et al. (1990) highlighted the role of genetic admixture in the initiation of population and species differentiation. They suggested that within species, the juxtaposition of different genetic combinations would be frequent during the growth phase of each of the Hawaiian volcanoes, declining as each

volcano becomes dormant, with the result that the youngest islands serve as evolutionary crucibles. This idea has been supported by small-scale studies in fragmented populations of flies (Carson and Johnson 1975; Carson et al. 1990) and spiders (Vandergast et al. 2004). However, because most of these examples have, over the longer history of the islands, also been associated with species proliferation, it has been impossible to tease apart the importance of such genetic effects within species from the process of speciation itself. The current study focuses on a single species, the Hawaiian happy-face spider *Theridion grallator* Simon (Araneae: Theridiidae), in which the genetic basis for adaptation to the environment differs between islands (Oxford and Gillespie 1996a) despite the ecological and phenotypic similarity of populations. As such, this species provides one of the best opportunities for examining the role of fragmentation and a shifting mosaic landscape in shaping the genetic structure of a taxon over the entire chronology of the archipelago.

Theridion grallator is a small spider that is largely restricted to wet and mesic forest on four of the Hawaiian Islands: Oahu, Molokai, Maui, and Hawaii. The similarity in genitalic characters with no obvious differences across islands and the viability of offspring from crosses between individuals from different islands (Oxford and Gillespie 1996b) together imply that this is a single species. The spider exhibits a dramatic color polymorphism for which more than 20 distinct color morphs have been described (Gillespie and Tabashnik 1989; Gillespie and Oxford 1998; Oxford and Gillespie 2001). Laboratory rearing experiments have indicated that this polymorphism is inherited in a Mendelian fashion through alleles at one (possibly two—see below) genetic locus, with the phenotypes exhibiting a dominance hierarchy that reflects the extent of expressed pigmentation (Oxford and Gillespie 1996a,b,c). In virtually all populations, the polymorphism comprises a common cryptic Yellow morph and numerous rarer patterned morphs, and appears to be maintained by balancing selection (Gillespie and Oxford 1998). Interestingly, a fundamental change occurred in the mechanism of inheritance of the color polymorphism on the island of Hawaii compared to Maui. On Hawaii, rather than being controlled by a single locus as on Maui, there is good evidence of a second locus being involved together with sex-limitation affecting several morphs (Oxford and Gillespie 1996a). In particular, the Red Front morph on Hawaii is restricted to males; females of the same genotype are Yellow (Oxford and Gillespie 1996a,b). This finding, together with data indicating high levels of differentiation among populations (Gillespie and Oxford 1998), suggests that the color polymorphism, or at least many of the rarer patterned morphs, may have been “reinvented” several times within the species.

The genetically based adaptive changes that have occurred between the highly structured populations of the Hawaiian happy-

face spider present a model system for examining evolutionary process within a shifting mosaic of isolation. Here, we set out to examine the effects of two processes on spider population structure. First, we explore the effect of population isolation, and the extent to which this is dictated by the mosaic structure of the landscape both within and among islands. Second, we assess the extent to which cycles of expansion and contraction of the habitat matrix are associated with population expansion and contraction in *T. grallator*. These two processes are explored with mitochondrial and nuclear markers, classical population genetic estimates of diversity, Bayesian coalescent phylogenetic and dating analyses, and coalescent-based inferences of likely migration models. The population genetic structure is examined in the context of the known genetic bases for color polymorphism in the different populations.

Materials and Methods

SAMPLE COLLECTION

Theridion grallator was collected directly from the undersides of broad-leaved plants such as *Broussaisia arguta* (Saxifragaceae) and *Clermontia arborescens* (Campanulaceae). Collection location details are given in Figure 1. For numbers of individuals collected and subject to different analyses see Table 1. Throughout this article, sites are referred to by their location numbers, for example [3]. Specimens were collected in 1998 (allozyme samples) and between 2008 and 2010 (mtDNA samples, except Kamakou, Molokai [2] and Puu Kukui, West Maui [3] from allozyme extractions).

The analysis was conducted at two levels. We distinguished the samples by the volcano on which they were collected. We also examined subpopulations on Waianae, Oahu (Puu Kaua [4d], and from three areas on Mt. Kaala [4a–c]), and on Haleakala, East Maui (Lower Waikamoi [4b], Upper Waikamoi [4a], Auwahi [4c]). Because populations of *T. grallator* are very patchily distributed and often at low densities, sample sizes were limited for sites [1d, 2, 3].

Sequences from *T. kauaiensis* and *T. posticatum* were employed as outgroups in the phylogenetic analysis as these were shown to be sister species to *T. grallator* (Arnedo et al. 2007).

ALLOZYME ELECTROPHORESIS

In a previous study (Gillespie and Oxford 1998), seven enzyme systems, yielding eight putative loci, were determined and scored in 107 specimens of *T. grallator* (four specimens from Kamakou, Molokai [2], 48 from Haleakala, Waikamoi, East Maui [4], 16 from Mt. Kohala, Hawaii [5], 24 from the Saddle, Hawaii [6], and 15 from Kilauea, Hawaii [7]—see Fig. 1) using cellulose acetate membrane electrophoresis, as part of a test for selection

Table 1. Summary statistics of molecular diversity by *Theridion grillator* population.

Population	n	Allozymes				Mitochondrial DNA			
		A	H _o	H _e	F _{IS}	n	n _p	λ	π _n
1. Waianae, Oahu	11	1.449	0.491	0.455	-0.091 ns	60	11	0.648	0.0024
1a. Mt. Kaala: Boardwalk	–	–	–	–	–	24	8	0.702	0.0032
1b. Mt. Kaala: Camp	–	–	–	–	–	13	2	0.154	0.0024
1c. Mt. Kaala: Substation	–	–	–	–	–	20	6	0.716	0.0026
1d. Puu Kaua	–	–	–	–	–	3	1	0.000	0.0000
2. Kamakou, Molokai	4	1.597	0.550	0.533	0.043 ns	4	2	0.500	0.0075
3. Puu Kukui, West Maui	8	1.637	0.325	0.407	0.213 ns	8	2	0.536	0.0004
4. Haleakala, East Maui	48	2.004	0.413	0.485	0.150***	123	27	0.907	0.0077
4a. Upper Waikamoi	–	–	–	–	–	28	8	0.754	0.0063
4b. Lower Waikamoi	–	–	–	–	–	86	22	0.904	0.0086
4c. Auwahi	–	–	–	–	–	9	1	0.000	0.0000
5. Mt. Kohala, Hawaii	16	1.702	0.375	0.402	0.068 ns	17	10	0.904	0.0063
6. Saddle, Hawaii	24	1.842	0.464	0.437	-0.062 ns	64	22	0.824	0.0053
7. Kilauea, Hawaii	15	1.966	0.338	0.480	0.313**	55	5	0.236	0.0013
Mean		1.794	0.422	0.457	0.091 ns		11.286	0.651	0.0044
SD		0.239	0.083	0.047	0.145		9.793	0.248	0.0030

n, number of individuals; A, mean allelic richness; H_o, observed heterozygosity; H_e, expected heterozygosity (corrected for small sample size); F_{IS}, Wright's inbreeding coefficient; n_p, number of haplotypes observed; λ, haplotype diversity; π_n, nucleotide diversity. Significance levels for F_{IS} values: *P < 0.05; **P < 0.01; ***P < 0.001; ns, not significant.

operating on the color polymorphism displayed by *T. grillator*. Here, we reanalyze these data, together with previously unanalyzed data from this collection (an additional eight specimens from Puu Kukui, West Maui [3] and 11 specimens from Waianae, Oahu [Mt. Kaala Boardwalk {1a}]). Enzymes and allele frequencies are documented in Table S1.

DNA ISOLATION AND SEQUENCING

DNA was extracted from 331 specimens of *T. grillator* and two fragments of the mtDNA were sequenced: a 658-bp fragment of the *cytochrome c oxidase* subunit 1 (*COI*) gene and a 612-bp region encompassing part of the large ribosomal subunit (*16SrRNA*), the *tRNA^{Leu}^{CUN}* gene, and 407 bp of the *NADH* subunit 1 (*ND1*) gene. (For full PCR and sequencing conditions see Supporting Information.)

STATISTICAL ANALYSES

DNA sequence analysis

DNA sequence traces were edited using SEQUENCHER version 4.6 (GeneCodes, Ann Arbor, MI) and aligned and manipulated using MESQUITE version 2.73 (Maddison and Maddison 2009) and CLUSTAL X version 2.0 (Larkin et al. 2007). All sequences were examined to ensure that they were not pseudogenes. Summary statistics of genetic diversity among mtDNA haplotypes within populations were calculated using ARLEQUIN version 3.5 (Excoffier and Lischer 2010). These included haplotype diversity

λ (Nei 1987) and nucleotide diversity averaged over all sites π_n (Tajima 1983).

Population structure among islands, volcanoes, and among subpopulations within volcanoes was analyzed in two ways. First, analysis of molecular variance (AMOVA) (Excoffier et al. 1992; Excoffier and Smouse 1994) was performed using ARLEQUIN version 3.5 (Excoffier and Lischer 2010) to apportion genetic variation into within- and among-population, and among-island components. Second, pairwise genetic differentiation among populations was examined using AMOVA, as implemented by ARLEQUIN to estimate F_{ST} and Φ_{ST}—an analogue of Wright's F_{ST} that takes the evolutionary distance between individual haplotypes into account (Excoffier et al. 1992; Excoffier and Smouse 1994). For analyzing mtDNA subpopulation differentiation within Oahu [1] and East Maui [4], we chose to calculate F_{ST} (based only on haplotype frequencies), rather than Φ_{ST}, as new mutations were unlikely to have reached appreciable frequencies and genealogical relationships among haplotypes may be more likely to reflect historical events rather than extant population fragmentation (Vandergast et al. 2004). For all AMOVA-based analyses, significance was determined by 10,000 replicates, randomizing individuals over populations. Pairwise estimates of F_{ST} and Φ_{ST} were visualized through two-dimensional principal coordinates analysis (PCoA or multidimensional scaling) using the CMDSCALE function of R (R Development Core Team 2008). In addition, ARLEQUIN was used to estimate the “number of migrants per generation,” N_em_e according to the classic relationship with F_{ST} of

Wright (1951). The possible evolutionary relationships among the mtDNA haplotypes were reconstructed by generating phylogenetic networks using statistical parsimony (TCS version 1.13; Clement et al. 2000).

To identify the most likely routes of migration and colonization among islands and volcanoes, we followed a Bayesian framework for model choice that uses MIGRATE-N (Beerli and Felsenstein 2001; Beerli 2006) to choose among migration models on the basis of their ln marginal-likelihood. To manage the large number of possible models, we employed a modified stepwise approach (see Supporting Information).

Gene trees for the mtDNA data were constructed using BEAST version 1.6.0 (Drummond and Rambaut 2007). BEAST was used to simultaneously reconstruct gene trees and infer divergence times and mutation rates using the known ages of the Hawaiian Islands (Clague 1996, see Fig. 1) as node constraints. BEAST was also employed to examine the historical demography of each volcano population and island using Bayesian skyline plots (Drummond et al. 2005). (For full details see Supporting Information).

Recent departures from a constant population size were examined using the mtDNA data and the summary statistics F_s (Fu 1997) and Tajima's D (Tajima 1989). Large negative values of F_s indicate population growth. Tajima's D not only has good power to detect population growth (Ramos-Onsins and Rozas 2002) (or departures from neutrality), but offers a two-tailed test: significantly negative values of Tajima's D indicate population expansion (following a population bottleneck or a selective sweep) and significantly positive values indicate population contraction or genetic subdivision (or diversifying selection) (Eytan and Hellberg 2010). Statistical significance was assessed by comparing the observed values to the distribution of 10,000 coalescent simulations in ARLEQUIN version 3.5 (Excoffier and Lischer 2010). Finally, the moment estimator of time since a sudden demographic expansion τ was estimated from the mtDNA mismatch distribution using ARLEQUIN version 3.5 (Excoffier and Lischer 2010), with 95% confidence intervals estimated from 10,000 bootstrap replicates. This was used to estimate t , using the mutation rate inferred by BEAST, as an additional indicator of initial colonization times.

Allozyme analysis

For each population, allozyme allele frequencies per locus, the mean allelic richness (A), based upon the rarefaction method of El Mousadik and Petit (1996), the observed heterozygosity (H_o), the expected heterozygosity (H_e) corrected for small sample size (Nei 1978), and Wright's inbreeding coefficient (F_{IS}) corrected for small sample size (Kirby 1975) were calculated using FSTAT version 2.9.3.2 (Goudet 1995). Tests for genotypic disequilibrium between pairs of loci and tests for deviations from Hardy-Weinberg

equilibrium for each population and locus were performed using randomization tests (10,000 realizations) as implemented by FSTAT with sequential Bonferroni-type correction (Rice 1989).

AMOVA analyses of the allozyme data (F_{ST} -based) were calculated as for the mtDNA sequence data except that estimates were averaged across loci. Pairwise estimates of F_{ST} were also similarly visualized through PCoA.

Bayesian clustering analyses were applied to the allozyme data using STRUCTURE version 2.3 (Pritchard et al. 2000) to detect differentiated populations without the need to define populations a priori. The admixture model (k) with uncorrelated allele frequencies was employed, with sampling locality as a prior (Hubisz et al. 2009). The use of this prior does not lead to a tendency to find population structure when none is present, but can improve the ability of the algorithm to detect structure when data are limited (Hubisz et al. 2009). Allele frequencies tended to be skewed toward low/high frequencies in each population, therefore an optimal value for λ , the allele frequency prior, was inferred by running 10 replicate analyses at $k = 1$, yielding $\bar{\lambda} = 0.6466$. Ten replicate runs were then performed for k values between 1 through to 15. All runs employed a burn-in of 100,000 iterations and a sample of 100,000 iterations. STRUCTURE HARVESTER (Earl 2009) was used to summarize the ln probability of the data for each replicate at each value of k (ln Pr($X|K$)). Output files for the optimum estimate of k were merged in CLUMPP (Jakobsson and Rosenberg 2007) and graphed using DISTRUCT (Rosenberg 2004).

The most likely routes of colonization among islands and volcanoes were evaluated using MIGRATE-N-based model-choice (Beerli and Palczewski 2010), similar to the mtDNA analyses (see Supporting Information).

BOTTLENECK (Piry et al. 1999) was employed to look for evidence of recent bottlenecks from the within-population allozyme allele frequency data. This test is based upon the theoretical expectation that in a recently reduced population, the number of alleles is reduced more quickly than the expected heterozygosity (H_e). Hence, following a recent bottleneck, H_e should exceed the equilibrium heterozygosity (H_{eq}), as estimated by a coalescent process from the observed number of alleles, under the assumption of mutation-drift equilibrium (Cornuet and Luikart 1996; Luikart and Cornuet 1998). As recommended for allozyme data and a small number of loci (Piry et al. 1999), the data were analyzed under the Infinite Alleles Model (IAM) and employed Wilcoxon signed-rank tests to evaluate significance. To evaluate H_{eq} , 100,000 coalescent simulations were run. For comparison, this test was also applied to the mtDNA haplotype data, ignoring evolutionary distances between haplotypes and simply using the within-population haplotype frequencies as allele data.

Results

ALLELIC AND HAPLOTYPIC VARIATION

Of the eight allozyme loci, the number that was polymorphic per population ranged from four to eight. A total of 38 alleles were detected: *6Pgdh* (four alleles), *Mdh-1* (four alleles), *Idh-1* (three alleles), *Pgm-1* (five alleles), *Pgi-1* (eight alleles), *Gpdh-1* (four alleles), *Gpdh-2* (two alleles), *Mpi-1* (eight alleles). Allele frequencies per enzyme and population are given in Table S1. No linkage disequilibrium between pairs of loci was found after sequential Bonferroni-type correction. Mean values for the estimates of within-volcano population genetic variation for the allozyme data are given in Table 1. Allelic richness (A) ranged from 1.50 (Waianae [1abc], see Fig. 1) to 2.00 (Haleakala [4ab]), and observed heterozygosity (H_o) ranged from 0.34 (Kilauea [7]) to 0.55 (Kamakou [2]) and 0.49 (Waianae [1abc]). Expected heterozygosity (H_e , corrected for small sample size) ranged from 0.40 (Mt. Kohala [5]) to 0.53 (Kamakou [2]). Wright's inbreeding coefficient F_{IS} ranged from -0.09 (Waianae [1abc]) to 0.31 (Kilauea [7]). Only two populations (Haleakala [4ab] and Kilauea [7]) showed significant deviations from Hardy-Weinberg expectations and in both cases they exhibited significantly positive F_{IS} values (i.e., deficiency of heterozygotes), however neither remained significant after Bonferroni-type correction. No individual loci showed significant deviations from Hardy-Weinberg expectations after Bonferroni-type correction.

A total of 73 mtDNA haplotypes (1270 bp of *COI* and *16S-ND1* sequences combined) (Table S2) were identified among a sample of 331 *T. gallator* specimens. Alignment to database sequences indicated no stop-codons, insertions, or deletions that would indicate evidence of pseudogenes. A total of 44 substitutions were observed of which 38 were transitions and six were transversions. The sequences were AT rich (75.61%). Overall, 42 (57.53%) of the haplotypes were observed only once. The nine most common haplotypes (observed 10 or more times) accounted for 61.14% of the individuals. No haplotypes were shared among Oahu, Greater Maui, or Hawaii, and no haplotypes were shared among the volcanoes of Greater Maui, with the exception of one (MA_05) common to Kamakou [2] and Haleakala: Lower Waikamoi [4b]. Within Hawaii, only four haplotypes (HA_01, HA_02, HA_03, HA_06) of 32 were shared among populations (see Table S2). Even so, haplotype diversity was generally high within individual volcano populations, whereas nucleotide diversity was low (Table 1). Maximal haplotype diversities were observed in Haleakala: Lower Waikamoi [4b] $\lambda = 0.90$ ($\pi_n = 0.009$), and on Mt. Kohala [5], $\lambda = 0.90$ ($\pi_n = 0.006$). The lowest haplotype diversity was on the youngest volcano, Kilauea [7] where only five haplotypes were observed in 55 individuals ($\lambda = 0.24$; $\pi_n = 0.001$), with one (HA_01) accounting for 48 (87.27%) of the individuals.

POPULATION GENETIC STRUCTURE

Table 2 shows the results of AMOVA analyses of population structure, pairwise estimates of F_{ST} and Φ_{ST} and corresponding estimates of $N_e m_e$. For the mtDNA data, AMOVA analyses using Φ_{ST} among islands and among volcano populations (Table 2A) revealed extremely strong structuring with 61.37% of mtDNA variation occurring among islands, 14.20% of variation occurring among populations within islands, and 24.44% occurring within populations. When the analysis was performed using only haplotype frequencies (traditional F_{ST} -based approach—excluding the among island category as no haplotypes were shared among islands), significant structure was still evident with 28.82% of the variation occurring among populations and 72.74% occurring within populations (Table 2A). The relative differences in the proportions of variation between the Φ_{ST} and F_{ST} estimates (within populations: $\Phi_{ST} = 24.44\%$, $F_{ST} = 72.74\%$; Φ_{ST} among all populations: [among islands + among population within islands] = 75.57%, $F_{ST} = 28.82\%$) result from the evolutionary distance information used in the calculation of Φ_{ST} and the lack of shared haplotypes among islands. Overall Φ_{ST} (0.76) and F_{ST} (0.29) estimates were highly significant ($P < 0.00001$). Pairwise estimates of Φ_{ST} were also generally very high, with all values (excluding comparisons among the populations within Hawaii and with the exception of Haleakala [4]–Molokai [2] [$\Phi_{ST} = 0.37$]) exceeding 0.55. Pairwise values of Φ_{ST} among the populations on Hawaii were lower, notably that between the Saddle [6] and Mt. Kohala [5] ($\Phi_{ST} = 0.09$, $P = 0.0071$). With the exception of the latter, P -values for all comparisons were less than 0.001. Correspondingly, estimates of $N_e m_e$ were also extremely low, with all values < 1 with the exception of Saddle [6] and Mt. Kohala [5] ($N_e m_e = 5.26$), suggesting very little movement between volcano populations.

AMOVA analyses of the allozyme data (Table 2B) revealed a similar picture to that of the F_{ST} -based mtDNA AMOVA, with 20.60% of variation occurring among islands, 12.65% occurring among populations within islands (i.e., 33.25% among all populations), and 66.75% occurring within populations. Overall F_{ST} was highly significant ($F_{ST} = 0.33$, $P < 0.00001$). Pairwise estimates of F_{ST} were also high with most comparisons yielding values greater than 0.40 and P -values less than 0.001. Again, estimates among the populations on Hawaii were much lower, notably between Mt. Kohala [5] and Kilauea [7] ($F_{ST} = 0.04$, $P = 0.0428$) and between the Saddle [6] and Kilauea [7] ($F_{ST} = 0.03$, $P = 0.0311$). Pairwise estimates of F_{ST} between Haleakala [4ab] and all other volcano populations were intermediate (range 0.14–0.34) with corresponding estimates of $N_e m_e > 1$ (with the exception of Haleakala [4ab]–Mt. Kohala [5], $N_e m_e = 0.97$), which might suggest an intermediary role for Haleakala (East Maui) in the colonization of other islands. In general, though, estimates of $N_e m_e$ were less than one.

Table 2. Pairwise estimates of population divergence and migration among *Theridion grallator* populations. Φ_{ST} given below the diagonal, $N_e m_e$ given above the diagonal.

A. mtDNA by volcano (Φ_{ST})							
	1.	2.	3.	4.	5.	6.	7.
1. Waianae, Oahu	–	0.0702	0.0492	0.1549	0.0519	0.0952	0.1110
2. Kamakou, Molokai	0.8769	–	0.1154	0.8434	0.0694	0.3824	0.3025
3. Puu Kukui, West Maui	0.9105	0.8125	–	0.3966	0.0444	0.1848	0.1997
4. Haleakala, East Maui	0.7635	0.3722	0.5577	–	0.1987	0.3291	0.2975
5. Mt. Kohala, Hawaii	0.8401	0.5666	0.7302	0.6031	–	5.2578	0.7872
6. Saddle, Hawaii	0.8184	0.6231	0.7146	0.6270	0.0868	–	0.9430
7. Kilauea, Hawaii	0.9060	0.8781	0.9185	0.7159	0.3885	0.3465	–
AMOVA (F_{ST} and Φ_{ST} -based)	F_{ST}		Φ_{ST}				
% Variation among islands	NA		61.37				
% Variation among populations (within islands) ²	28.82		14.20				
% Variation within populations	71.18		24.44				
Overall F_{ST} / Φ_{ST} (P -value)	0.2882 ($P < 0.00001$)		0.7556 ($P < 0.00001$)				
B. Allozymes by volcano (F_{ST})							
	1.	2.	3.	4.	5.	6.	7.
1. Waianae, Oahu	–	0.5967	0.4077	2.3568	0.3760	0.4850	0.4690
2. Kamakou, Molokai	0.4559	–	0.5833	3.0659	0.6487	0.8288	0.7077
3. Puu Kukui, West Maui	0.5508	0.4616	–	1.2257	0.5522	0.4967	0.6368
4. Haleakala, East Maui	0.1750	0.1402	0.2897	–	0.9652	1.2941	1.5008
5. Mt. Kohala, Hawaii	0.5708	0.4352	0.4752	0.3412	–	2.7070	11.3581
6. Saddle, Hawaii	0.5076	0.3763	0.5017	0.2787	0.1559	–	14.5489
7. Kilauea, Hawaii	0.5160	0.4140	0.4399	0.2499	0.0422	0.0332	–
AMOVA (F_{ST} -based)							
% Variation among islands	20.60						
% Variation among populations (within islands)	12.65						
% Variation within populations	66.75						
Overall F_{ST} (P -value)	0.3325 ($P < 0.00001$)						

Continued.

Table 2. Continued.

C. mtDNA East Maui, Haleakala subsamples (F_{ST})				
	4a.	4b.	4c.	
4a. Haleakala: Upper Waikamoi	–	4.8288	0.4898	
4b. Haleakala: Lower Waikamoi	0.0938	–	0.6860	
4c. Haleakala: Auwahi	0.5052	0.4216	–	
AMOVA (F_{ST} -based)				
% Variation among populations	26.88			
% Variation within populations	73.12			
Overall F_{ST} (P -value)	0.2688 ($P < 0.00001$)			
D. mtDNA Oahu, Waianae subsamples (F_{ST})				
	1a.	1b.	1c.	1d.
1a. Waianae: Mt. Kaala Boardwalk	–	3.1983	23.8013	0.5787
1b. Waianae: Mt. Kaala Camp	0.1352	–	2.7083	0.0731
1c. Waianae: Mt. Kaala Substation	0.0206 ¹	0.1559	–	0.6043
1d. Waianae: Puu Kaa	0.4635	0.8724	0.4528	–
AMOVA (F_{ST} -based)				
% Variation among populations	20.32			
% Variation within populations	79.68			
Overall F_{ST} (P -value)	0.2032 ($P < 0.00001$)			

¹ F_{ST} between the Boardwalk and the Substation (Oahu, Mt. Kaala) was not significant. ² F_{ST} (haplotype frequency based) AMOVA for mtDNA only computed at among and between population levels because no haplotypes were shared among island systems. Among islands refers to Oahu, Greater Maui (Molokai, West Maui and East Maui combined), and Hawaii.

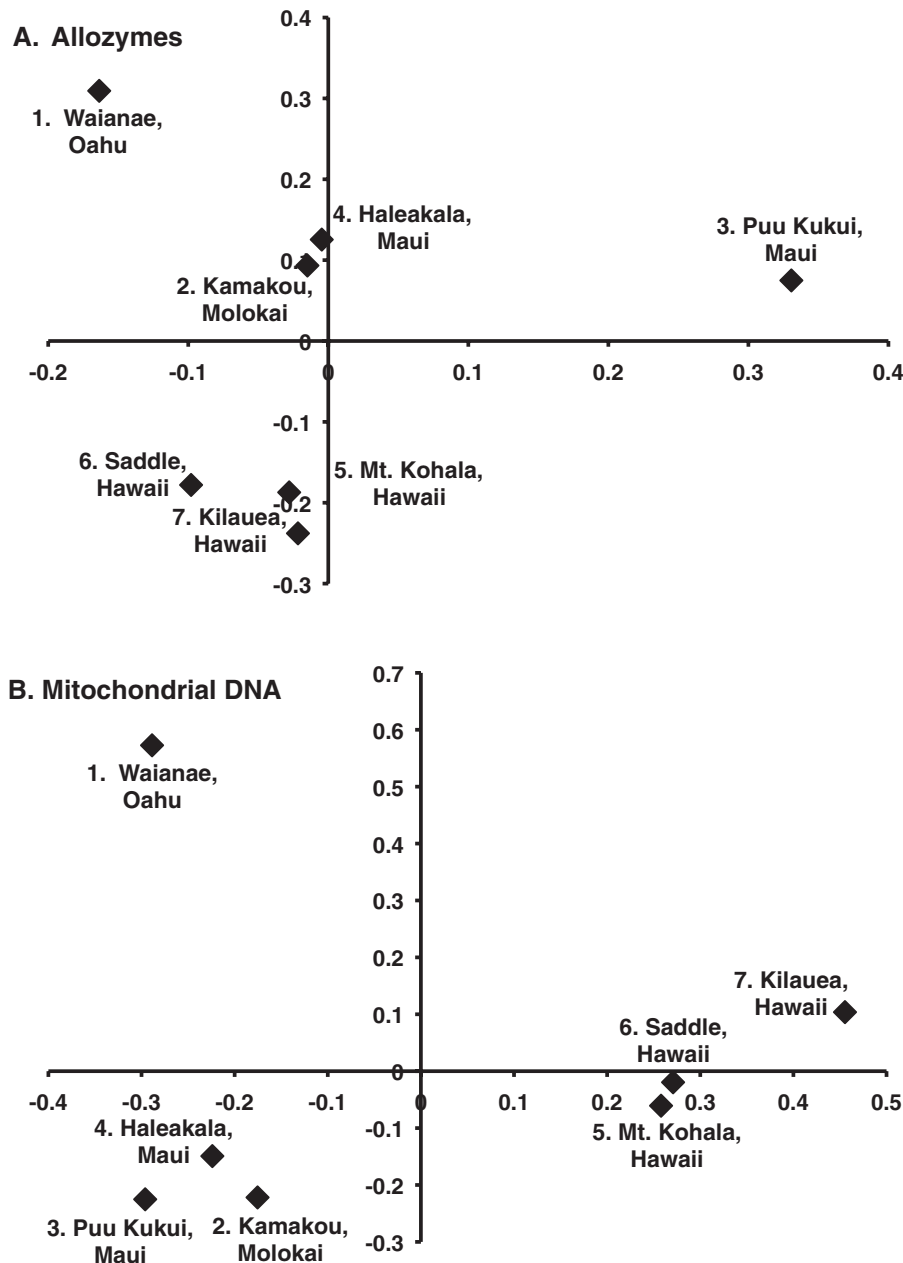


Figure 2. Principal coordinates analysis of population differentiation in two-dimensions. (A) Allozyme data— F_{ST} . (B) mtDNA data— Φ_{ST} .

Relationships among the volcano populations, based upon the matrices of F_{ST} (allozymes) and Φ_{ST} values (mtDNA), are graphically summarized using two-dimensional PCoA plots in Figure 2A and Figure 2B, respectively. The genetic relationships among the populations broadly reflect the geographical arrangement of the Hawaiian Islands. The allozyme data however suggest that Puu Kukui [3] is genetically distinct from the rest of Greater Maui (Haleakala [4ab] and Kamakou [2])—although note the small sample size ($n = 8$) for Puu Kukui [3].

AMOVA analyses, based upon mtDNA, among the subpopulations on Haleakala [4abc] and among the Waianae [1abcd]

subpopulations yielded surprisingly similar results to the among-volcano population analyses with 26.88% of variation on Haleakala [4abc] (Table 2C), and 20.32% of Waianae [1abcd] variation (Table 2D) occurring among populations. Pairwise estimates of F_{ST} among the Haleakala [4abcd] subpopulations were all highly significant ($P < 0.001$), although there was some evidence of more recent genetic exchange between Lower Wakamoi [4b] and Upper Waikamoi [4a], with an F_{ST} of only 0.09 and a corresponding estimate of $N_e m_e = 4.83$; this may not be surprising given the short distance (~ 4 km) and the continuous forest between these sites. Pairwise estimates of F_{ST} among the

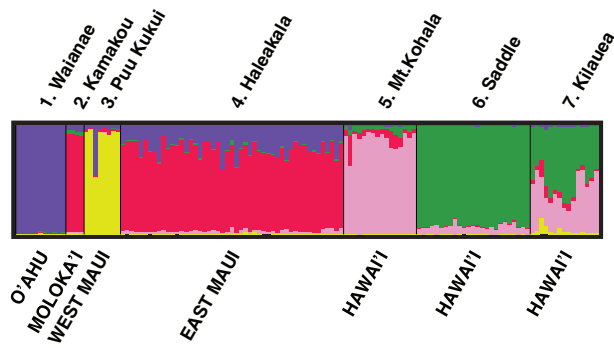


Figure 3. Graphical summary of results from the structure analysis of the allozyme data for $k = 5$ clusters. Each individual is represented by a vertical line broken into five colored segments representing the proportion of that individual's genome originating from the five clusters.

Waianae [1abcd] subpopulations, indicated that Puu Kaua [1d] was quite distinct from the other subpopulations [1abc] ($F_{ST} = 0.4528$ – 0.8724). Although the Mt. Kaala Boardwalk [1a] and Substation [1c] subpopulations were not significantly differentiated ($F_{ST} = 0.02$, $P = 0.1977$), these subpopulations were significantly differentiated from the Camp [1b] subpopulation ($F_{ST} = 0.14$, $P = 0.0259$ and $F_{ST} = 0.16$, $P = 0.0124$, respectively). Each Mt. Kaala subpopulation [1abc] was <1 km apart (Fig. 1).

Plots of the ln probability of the data ($\ln \Pr(X|K)$) for each replicate at each value of k in the STRUCTURE analyses of the allozyme data clearly indicated a peak in $\ln \Pr(X|K)$ at $k = 5$. This value was therefore chosen as the best estimate for the number of genetic subpopulations among the volcano populations. The results of the STRUCTURE analysis at $k = 5$, averaged over 10 replicate runs, are given in Figure 3. These results concur with the ordination in Figure 2A, illustrating the genetic similarity between Haleakala [4abc] and Kamakou [2]. These in turn show some affinity with the Waianae populations [1abc]. Puu Kukui [3] is genetically distinct from the other Greater Maui populations. Overall, the Hawaii populations are genetically distinct from the Greater Maui and Oahu populations. The Mt. Kohala [5] population is quite distinct from that on the Saddle [6] whereas the Kilauea [7] population appears to be intermediate between the two.

MITOCHONDRIAL HAPLOTYPE RELATIONSHIPS

Figure 4 shows mtDNA haplotype networks for Oahu (Fig. 4A), Greater Maui (Fig. 4B), and Hawaii (Fig. 4C). Networks were constructed separately for each island system for clarity and because no haplotypes were shared among islands. The outgroup taxa, *T. positticatum* and *T. kauaiensis* were included in the Oahu network as they were most similar to the Oahu *T. grallator* haplotypes. The outgroups connect with the Puu Kaua [1d] haplotype (OA_11), suggesting that this haplotype may be more ancestral than the other Waianae haplotypes. Overall, the Waianae: Mt Kaala [1abc]

haplotypes show little structuring, as expected given that they represent subsites within a single locality. Within Greater Maui (Fig. 4B), most haplotypes came from Haleakala: Waikamoi [4ab], and with the exception of the distinct Kamakou [2] and Puu Kukui [3] haplotypes, again show little obvious structure. The single haplotype from Auwahi [4c] (MA_01, labeled “A” in Fig. 4B) is most similar to the common Waikamoi [4ab] haplotype MA_06 from the same volcano (Haleakala). In Hawaii (Fig. 4C), numerous haplotypes from the Saddle [6] and Mt. Kohala [5] are scattered throughout the network. The Kilauea [7] haplotypes were more clustered, perhaps reflecting a recent origin from few founding haplotypes.

COLONIZATION AND MIGRATION MODELS

MIGRATE-N was employed to determine the most probable models of colonization among the seven *T. grallator* volcano populations. Previous phylogenetic analyses (Arnedo et al. 2007) and the structure, network, and phylogeographic analyses presented here (see below) all indicate that Oahu was the most likely source of all extant *T. grallator* populations and that colonization most likely occurred from Oahu to Greater Maui to Hawaii. Furthermore, the extremely high estimates of F_{ST} and Φ_{ST} , and correspondingly low estimates of $N_e m_e$, among islands (Table 2) suggest that very little exchange has occurred among islands. The MIGRATE-N analyses strongly supported this pattern and the most likely full migration model (allowing both unidirectional and back-migration), together with MIGRATE-N-based $N_e m_e$ estimates, is illustrated in Figure 5. (The top five models for the mtDNA and allozyme data, together with their corresponding *LBFs* are illustrated in Fig. S1.) Both the mtDNA data and the allozyme data yielded similar models (Figs. 5 and S1). The only differences were that the best allozyme model did not support migration from Puu Kukui [3] to Kamakou [2], or from Kilauea [7] to the Saddle [6] (as indicated by the best mtDNA model), and the mtDNA data did not support migration from the Saddle [6] to Mt. Kohala [5], or from the Saddle [6] to Kilauea [7] (as indicated by the best allozyme model). Estimates of $N_e m_e$, computed for the combined mtDNA and allozyme model (Fig. 5) using MIGRATE-N, were low, as expected from the AMOVA-based estimates. Estimates of $N_e m_e$ between Oahu and Greater Maui and between Greater Maui and Hawaii were particularly low.

PHYLOGENETIC DATING

Figure S2 shows a 50% majority rule gene-tree constructed from 20,000 posterior trees output by BEAST. This tree (and also the chronogram in Fig. 6; see below) shows the monophyly, and apparent independent evolution, of mtDNA haplotypes on each major island group (Oahu, Greater Maui, and Hawaii). The tree also clearly shows the progression of colonization from Oahu, to Greater Maui, to Hawaii. However, although posterior

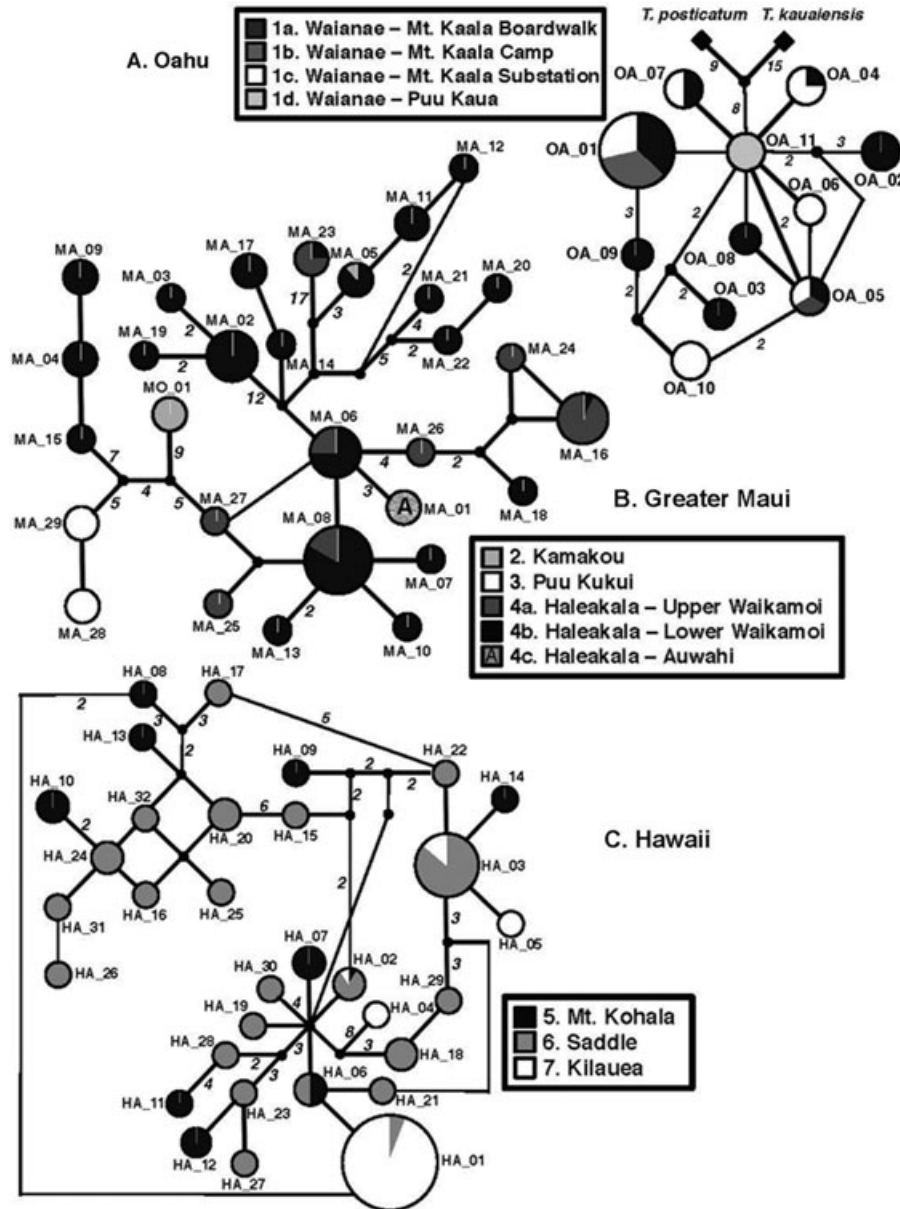


Figure 4. Haplotype networks for Oahu, Greater Maui, and Hawaii, constructed using a statistical parsimony algorithm with the program rcs version 1.13 (Clement et al. 2000). Sampled haplotypes are designated with an island code (OA, Oahu; MA, Maui; HA, Hawaii) and inferred missing haplotypes are indicated as black dots (nodes). Numbers next to the branches indicate the number of substitutions (if > 1) occurring on that branch. Larger circles represent more common haplotypes and pie diagrams indicate the frequency of each haplotype by population. The single haplotype from Auwahi, East Maui has been marked with an "A" for clarity.

probabilities for most major clades were high (1.00), the support for the Maui-Hawaii split was only 0.51—perhaps reflecting the fact that Hawaii and Greater Maui were colonized during a similar time-period (see below).

A chronogram was constructed using posterior means from BEAST (Fig. 6). Divergence intervals, in Ma, together with their 95% credible intervals (95% CIs) are indicated on this figure. The 95% CIs were broad, however the results do suggest the probable order of colonization events. The root of the chronogram

was located at 4.22 Ma (0.95–8.25 Ma), an interval that spans both the emergence of Oahu (3.70 Ma) and of Kauai (5.10 Ma). Although a date of 4.22 Ma suggests that *T. grallator* diverged from the outgroups on Kauai (where the species has not been found), we cannot be certain of its exact origins within the islands. Colonization of the islands currently inhabited by *T. grallator* appears to have happened much more recently, with the split between Oahu and Greater Maui (and Hawaii) suggested as being around 0.78 (0.37–1.34 Ma), an interval during which the entirety

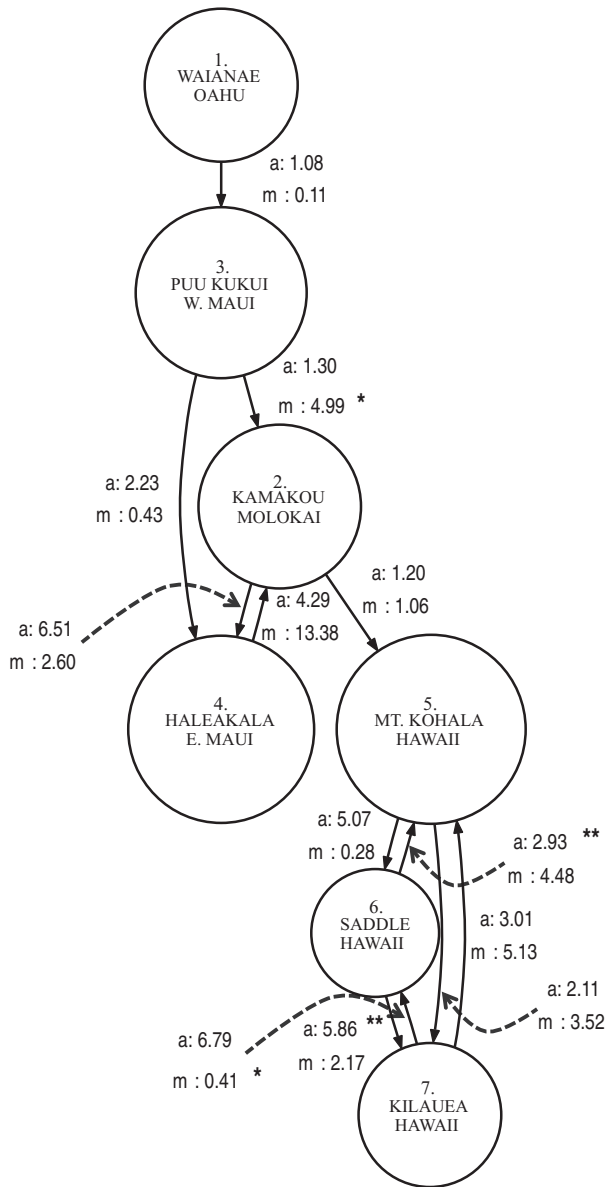


Figure 5. Migration routes of *Theridion grallator* among Hawaiian volcano populations as selected by a Bayesian model choice procedure utilizing Migrate-n. Migration/colonization routes are shown by solid arrows. Estimates of $N_e m_e$ from Migrate-n are given beside each arrow (a, allozyme; m, mtDNA; *connection not inferred by allozyme data; **connection not inferred by mtDNA data).

of Greater Maui would have already emerged above sea level. The split between Greater Maui and Hawaii was placed at 0.56 (0.37–0.75 Ma), an interval that overlaps extensively with the split between Oahu and Greater Maui (and slightly exceeds the oldest current age estimate for Hawaii of 0.43 Ma)—suggesting that the colonization of Greater Maui and Hawaii happened within a similar time-period.

DEMOGRAPHIC CHANGES

Summary statistics that aim to detect recent population changes are given in Table 3. For the allozyme data, BOTTLENECK revealed an excess of loci showing greater than expected levels of heterozygosity ($H_e > H_{eq}$) under IAM in six of the seven populations analyzed. The Wilcoxon signed-rank tests achieved statistical significance for two of the populations: Kamakou [2] ($P = 0.047$) and the Saddle [6] ($P = 0.037$). The Waianae [1abc] population also came close to significance ($P = 0.063$). When the P -values for the independent populations were combined using Fisher’s method (Fisher 1932), the overall test was significant ($\chi^2 = 26.09$; $df = 12$, $P = 0.0104$), suggesting that some, if not most, of the populations have undergone a recent decline in effective population size. The mtDNA data suggested a different story. BOTTLENECK analyses, treating the mitochondrial haplotype as alleles, suggested that six of the seven volcano populations showed a deficit of heterozygosity ($H_e < H_{eq}$) under IAM. Significance testing (the probability of the observed difference in heterozygosity divided by the SD, tested against the neutral model because there was only one locus) indicated that this deficit was significant for Waianae [1] ($P = 0.044$), the Saddle [6] ($P = 0.005$), and Kilauea [7] ($P = 0.030$). Fisher’s combined P -value was highly significant ($\chi^2 = 31.37$; $df = 12$, $P = 0.0017$). The Kilauea [7] population also showed a significantly negative value of Tajima’s D (Tajima’s $D = -1.77$, $P = 0.019$). Although six of the seven volcano populations also exhibited negative values of Tajima’s D , none of these achieved significance. Fisher’s combined P -value was however significant ($\chi^2 = 23.30$; $df = 12$, $P = 0.0253$). Three of the seven volcano populations exhibited negative values of Fu’s F_s , however none of these approached significance and the Fisher’s combined P -value was not significant ($\chi^2 = 8.63$; $df = 12$, $P = 0.7346$). These demographic summary statistics from the allozyme and mtDNA data therefore appear to contradict each other, with the allozyme data generally suggesting recent population declines and the mtDNA data suggesting recent demographic expansions. The implications of this are discussed below.

Estimates of time since a “sudden demographic expansion” (suggestive of initial colonization times), inferred from the mtDNA mismatch distribution, are also given in Table 3 (both as τ and time, t , inferred as $t = \tau/2\mu$, using the mutation rate $\mu = 3.546 \times 10^{-8}$ substitutions/site/year as inferred by BEAST). The confidence intervals were broad and overlapping, especially for the smaller samples. Nonetheless, these estimates supported the logical progression of colonization in Hawaii from Mt. Kohala [5] ($t = 0.12$ [0.045–0.175] mya) to the Saddle [6] ($t = 0.080$ [0.008–0.192] mya) and then to the youngest volcano Kilauea [7] ($t = 0.033$ [0.004–0.039] mya). That Hawaii ($t = 0.086$ [0.022–0.172] mya) was colonized more recently than Greater Maui ($t = 0.196$ [0.095–0.277] mya) was also

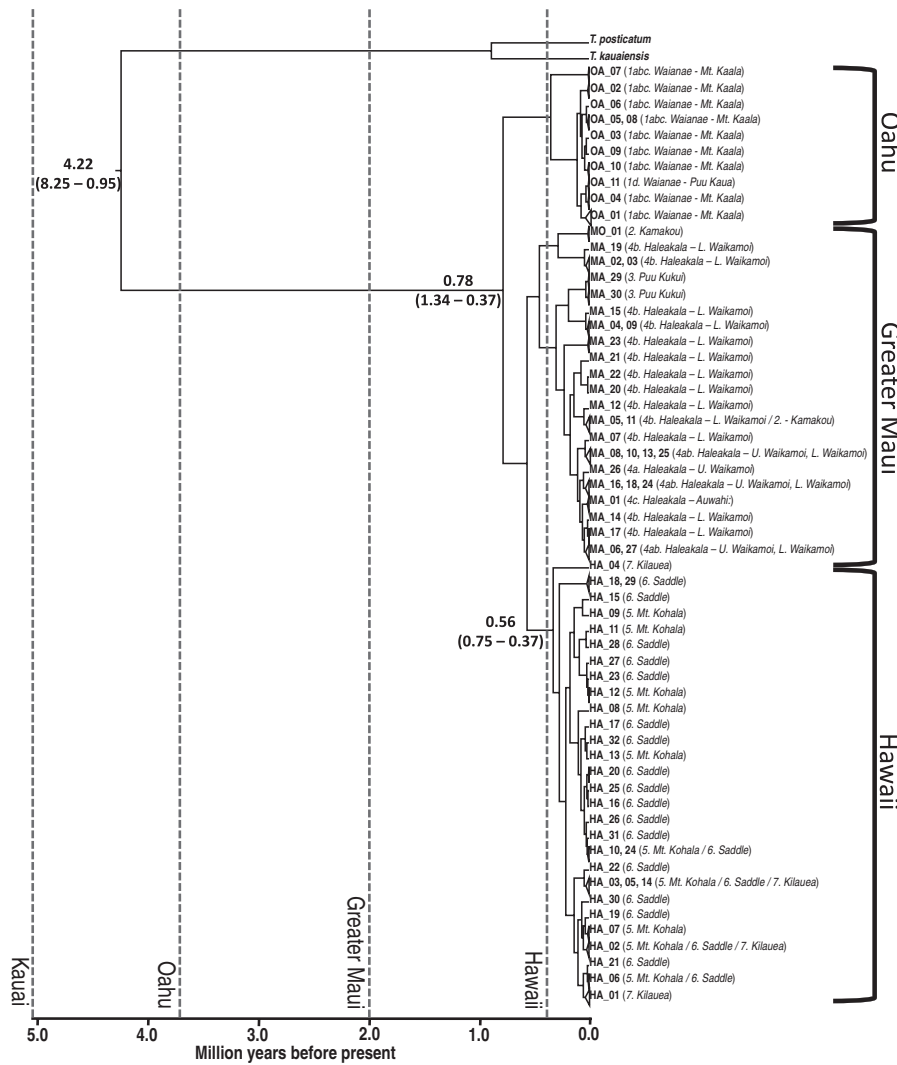


Figure 6. Chronogram constructed from 20,000 posterior trees output by BEAST. Divergence estimates and 95% credibility intervals (in parentheses) are indicated at the key nodes: Root, that is outgroups: *Theridion grallator*; Oahu: (Greater Maui + Hawaii); Greater Maui: Hawaii. Island ages are indicated by dashed-lines perpendicular to the time scale. The median rates of molecular evolution in units of substitutions/site/million years (95% credibility interval in parenthesis) were as follows: *CO1* coding region = 0.01704 (0.00803–0.02982); *16S-tRNA^{leu}^{CUN}* region = 0.05142 (0.01309–0.12020); *ND1* coding region = 0.03791 (0.01679–0.06801); mean overall rate = 0.03546.

supported. The colonization of Oahu appeared younger but with very broad confidence intervals ($t = 0.071$ [0.003–1.027] mya), probably reflecting the low haplotype diversity recorded from Waianae [1].

Bayesian coalescent skyline plots of the mtDNA data for Oahu, Greater Maui, and Hawaii are shown in Figure 7 (A–C). A complete set of plots for all populations is given in Figure S3. The plots for individual populations within Greater Maui and Hawaii were qualitatively very similar to the combined plots, perhaps because the mtDNA haplotypes within the major island systems (Oahu, Greater Maui, and Hawaii) tend to coalesce prior to the subpopulations on each island. No skyline plot showed statistically significant changes in effective population size. However, some interesting trends can be observed. First, in all populations a

notable decline in effective population size occurs around 10 Ka. In Greater Maui and Hawaii, this population decline is followed by a recent apparent increase in population size. The Hawaii plot (Fig. 7C) also shows a marked increase in population size beginning around 100 Ka. The same trend, albeit much weaker, is also evident in Greater Maui (Fig. 7B—note lower 95% CI) and to some extent in Oahu (Fig. 7A).

Discussion

HISTORICAL BIOGEOGRAPHY ACROSS THE HAWAIIAN ARCHIPELAGO

Phylogenetic analyses of *T. grallator* (mtDNA) confirmed that the species forms distinct monophyletic clades within each of the

Table 3. Results of summary statistic-based within-population demographic tests.

		Mitochondrial DNA											
Allozymes		$\pm H_e$		Bottleneck ¹		Tajima's <i>D</i>		Fu's <i>F_s</i>		τ ($\pm 95\%$ CI) ³		Time (mya) ($\pm 95\%$ CI) ³	
Bottleneck ²		$\pm H_e$		$\pm H_e$		$\pm H_e$		$\pm H_e$		$\pm H_e$		$\pm H_e$	
		$\pm H_e$	<i>P</i>	$\pm H_e$	<i>P</i>	$\pm H_e$	<i>P</i>	$\pm H_e$	<i>P</i>	$\pm H_e$	<i>P</i>	τ ($\pm 95\%$ CI) ³	Time (mya) ($\pm 95\%$ CI) ³
1. Waianae, Oahu	1/3	0.063	1/0	0.044	0.111	0.128	0.574	6.406	(0.250–92.406)	0.071	(0.003–1.027)		
1a. Mt. Kaala: Boardwalk	–	–	1/0	0.078	0.162	0.775	0.673	6.639	(0.391–12.676)	0.074	(0.004–0.141)		
1b. Mt. Kaala: Camp	–	–	1/0	0.367	0.057	0.362	0.343	3.000	(0.504–3.000)	0.033	(0.006–0.033)		
1c. Mt. Kaala: Substation	–	–	1/0	0.302	0.173	1.247	0.759	4.316	(0.592–8.006)	0.048	(0.007–0.089)		
1d. Puu Kaua	–	–	–	–	–	–	–	–	–	–	–	–	–
2. Kamakou, Molokai	1/4	0.047	1/0	1.000	0.072	5.524	0.984	0.000	(0.000–0.680)	0.000	(0.000–0.008)		
3. Puu Kukui, West Maui	3/3	0.922	0/1	0.287	0.927	0.866	0.571	0.832	(0.000–2.033)	0.009	(0.000–0.023)		
4. Haleakala, East Maui	2/6	0.231	1/0	0.212	0.394	–0.221	0.536	17.746	(4.508–27.279)	0.197	(0.050–0.303)		
4a. Waikamoi: Upper	–	–	1/0	0.247	0.704	4.067	0.939	23.203	(0.000–112.203)	0.258	(0.000–1.247)		
4b. Waikamoi: Lower	–	–	0/1	0.453	0.254	1.325	0.719	15.127	(5.133–21.871)	0.168	(0.057–0.243)		
4c. Auwahi	–	–	–	–	–	–	–	–	–	–	–	–	–
5. Mt. Kohala, Hawaii	2/5	0.289	1/0	0.383	0.323	–0.045	0.490	10.498	(4.084–15.783)	0.117	(0.045–0.175)		
6. Saddle, Hawaii	2/6	0.037	1/0	0.005	0.487	–2.930	0.196	7.203	(0.699–17.303)	0.080	(0.008–0.192)		
7. Kilauea, Hawaii	2/6	0.320	1/0	0.030	0.019	1.602	0.807	3.00	(0.504–3.500)	0.033	(0.004–0.039)		

¹ $\pm H_e$, number of loci exhibiting a deficit/excess of heterozygotes ($H_e < /> H_{eq}$). *P*-value from Wilcoxon signed-rank tests.

² $\pm H_e$, mtDNA alleles (haplotypes) exhibiting a deficit/excess of heterozygotes ($H_e < /> H_{eq}$). *P*-value from $\Pr(|DH|/SD < /> H_{eq})$.

³Time *t* since “sudden demographic expansion” was estimated as $t = \tau/2\mu$, using the mutation rate $\mu = 3.546 \times 10^{-8}$ substitutions/site/year (for 1270-bp mtDNA = 4.503×10^{-5}) as inferred by BEAST. Values were also estimated for Greater Maui ($\tau = 17.625$ [7.621–24.908]; $t = 0.196$ [0.095–0.277]) and Hawaii ($\tau = 7.795$ [1.971–15.459]; $t = 0.086$ [0.022–0.172]).

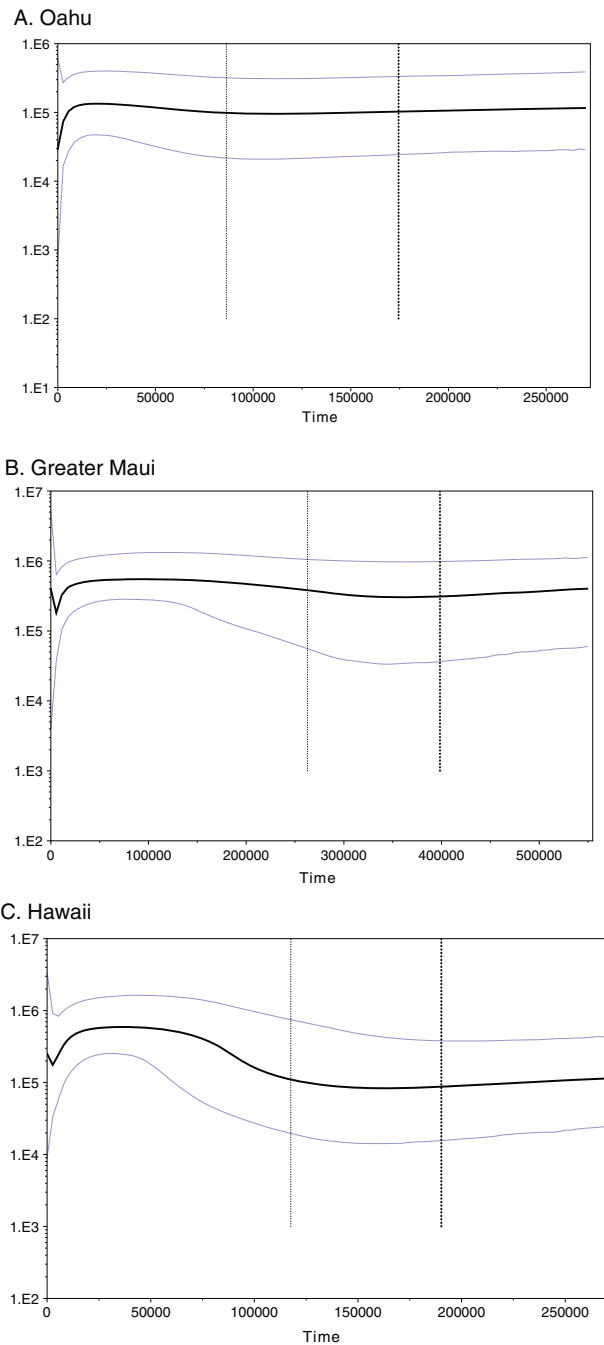


Figure 7. Bayesian skyline plots for the mtDNA data for populations of *Theridion grallator*. Time in years is shown on the x-axis and effective population size in log number of individuals is shown on the y-axis. The central dark horizontal line in each plot is the median value for the effective population size over time. The light lines are the upper and lower 95% credibility intervals (HPD) for those estimates. The far right of the plot is the upper 95% CI for the time to the most recent common ancestor (TMRCA). The vertical line to the left of this the median TMRCA and the vertical line to the left of that is the lower 95% CI for the TMRCA. For a complete set of plots for each volcano population, see Fig. S3.

major island groups, suggesting extremely little current exchange of individuals among islands. The order of colonization fits the “progression rule” (Funk and Wagner 1995), with younger islands being colonized sequentially from older islands. This order of events was confirmed through Bayesian migration model choice using both the mtDNA and allozyme data and also indicated by the estimates of initial colonization time inferred from the mtDNA mismatch distributions.

The relaxed molecular clock estimates suggest that *T. grallator* diverged from its ancestors approximately 4.22 Ma (0.95–8.25 Ma) (Fig. 6). Although our chronology suggests an older date for the colonization of Greater Maui from Oahu (0.78 Ma) than for the colonization of Hawaii from Greater Maui (0.56 Ma), the confidence intervals for these estimates overlap. These dates suggest that the colonization of Greater Maui and subsequently of Hawaii, from Oahu, occurred rapidly and recently relative to the age of the islands. Rapid colonization of Greater Maui would not be unexpected because Greater Maui would still have comprised a single landmass at the time of colonization. Naturally, the estimates of divergence times are subject to the assumptions of using island ages as calibration points and are entirely based upon mtDNA. Although more (nuclear) loci would lead to greater precision in both the phylogeny and dating, this would not alleviate the errors introduced by assumed island ages and the lack of a calibration point external to the phylogeny. However, the relative timing of these events is likely to be consistent and the order of events agrees with that inferred from both the model choice analyses of the allozyme data and the mtDNA mismatch distributions. Indeed, the degree of agreement between the mtDNA and allozyme data in both colonization routes and migration estimates (AMOVA) is quite remarkable and strongly indicates that colonization of the different Hawaiian Islands by *T. grallator* has occurred through very rare and possibly extreme founder events.

Similar patterns of extreme structuring of populations within species that occur across multiple islands of the Hawaiian chain have been found in several other groups, including the spider *Tetragnatha quasimodo* (Roderick and Gillespie 1998). The elepaio flycatcher shows a comparable pattern with phylogenetic analyses indicating reciprocally monophyletic groups for each island on which it occurs (Vanderwerf et al. 2010). Likewise, *Drosophila grimshawi* (Piano et al. 1997) and two species of damselfly, *Megalagrion xanthomelas* and *M. pacificum*, are all highly structured between major island groups (Jordan et al. 2005), although show considerably more mixing of populations within islands (including Maui Nui). The land snail *Succinea caduca* also has populations that are structured across the islands, but evidence indicates somewhat more gene flow linking populations both within, and to some extent between, islands (Holland and

Cowie 2007). That populations within species in the Hawaiian Islands show a similar progression rule as is found at the species level suggests that niche pre-emption by already present members applies to populations as well as to species.

HISTORICAL BIOGEOGRAPHY AND DEMOGRAPHY AMONG VOLCANOES WITHIN ISLANDS

Among volcano populations, with the exception of some comparisons among those on Hawaii, all estimates of F_{ST} or Φ_{ST} were large and highly significant, with correspondingly small estimates for the “number of migrants per generation,” $N_e m_e$. Similarly, estimates of $N_e m_e$ from MIGRATE-N were also low. Although the assumptions of migration-drift equilibrium required for meaningful estimation of $N_e m_e$ are almost certainly violated in these analyses, the exact values are not important. The estimates strongly suggest that there is negligible gene flow among islands and little among volcano populations within islands. Even among subpopulations on the same volcano (Fig. 1; some < 1 km apart), gene flow is limited. BOTTLENECK analyses of the allozyme data suggest that most populations have undergone a recent decline in effective population size. The same test, treating mtDNA haplotypes as alleles, together with Tajima’s D suggests that many populations may have recently increased in size—in particular the Waianae [1], Saddle [6], and Kilauea [7] populations. For the latter two populations, this might reflect population growth following forest recovery from recent volcanic activity. The apparent discrepancies between the two types of data warrant further discussion. The deficit of heterozygotes against coalescent equilibrium expectations ($H_e > H_{eq}$) revealed by the allozyme analyses reflects the slightly positive F_{IS} values also observed in these data (significant prior to Bonferroni correction for Kilauea [7] and Haleakala [4]; Table 1). Rare colonization events and associated strong founder effects lead to the rapid loss of allelic diversity and an excess of expected heterozygosity. For *T. grallator*, this process is supported not only by between-island monophyly but also by the observation that within-population haplotype diversity was generally high, whereas nucleotide diversity was low (Table 1). This implies that independent founder effects result in unique populations that generally remain isolated long enough to accrue substitutions through mutation and drift (Holland and Cowie 2007). Successful founder events are followed by demographic expansion leading to a corresponding reduction of expected heterozygosity compared to neutral expectations. The smaller effective population size and higher mutation rate of the mtDNA data imply that it may track recent demographic changes more efficiently (but see below): with the mtDNA summary statistics tracking the post-founder effect population growth. That both the largest positive F_{IS} (Table 1) and the most significantly negative Tajima’s D (Table 3) were both recorded for the youngest population, Kilauea [7] ($t = 0.033$ [0.004–0.039] mya) is indicative of this process. How-

ever, *T. grallator* populations have also been shaped by current (on Hawaii) and historical volcanic activity, year-to-year fluctuations in precipitation, and long-term climatic changes (see below). Consequently, demographic oscillations, in conjunction with the differing effective population sizes and mutation rates of the nuclear and mtDNA markers, means that drift is likely to obfuscate these signals and decouple them so that neutral expectations are not met and demographic inferences from each type of marker are likely to disagree. Very similar patterns and processes have been described in the Hawaiian land snail *S. caduca* (Holland and Cowie 2007). However, it should be noted that the discrepancy between the allozyme and mtDNA data could also be the result of natural selection. Both allozymes (e.g., Nevo et al. 1986) and mtDNA (see Meiklejohn et al. 2007) may be subject to selection. For mtDNA, it has been suggested that frequent selective sweeps in larger populations may counteract the expected increase in genetic diversity, rendering mtDNA a poor indicator of population size (Meiklejohn et al. 2007).

Longer term demographic changes were assessed using Bayesian coalescent skyline plots. Although none of these showed “significant” changes in effective population size over time, they indicated a sudden drop in effective population size within the last 10,000 years. Although it is possible that these results are simply an artifact due to limited sampling of coalescent time points in recent history, the replicated appearance in plots for all three island systems, and in the plots for individual populations (Fig. S3), suggests that this is not likely. The observed drop in effective population size could be the result of several factors. First, and most interestingly, the decline coincides with a period of Holocene aridification in which C3 plants were replaced by C4 plants in the islands (Uchikawa et al. 2010). During cooler periods in the past, montane forest in Hawaii experienced drier conditions, whereas during warmer periods, montane forests were wetter, as evidenced by the high number of xerophytic plants at the end of the last glacial period 10,000 years ago and increasing hydrophytes during the temperature maximum 4000–6000 years bp (Nullett et al. 1998). In addition, the Hawaii plot (Fig. 7C) shows a marked increase in population size beginning around 100 Ka years, perhaps associated with the decreasing volcanic activity on the island, with associated increase in available habitat through vegetational succession. However, this time also agrees with the possible colonization time for Hawaii as indicated by mismatch distributions ($t = 0.086$ [0.022–0.172] mya), and may simply reflect expansion of the new population. Interestingly, this pattern matches that of other forest-dwelling spiders in Hawaii, in particular *Tetragnatha anuenue* and *T. brevignatha*. In both species, recent and rapid population expansion was found and suggested to reflect past fragmentation caused by lava flows followed by population expansion as individuals recolonized areas where forests had regenerated (Vandergast et al. 2004).

PATTERNS OF COLOR POLYMORPHISM INHERITANCE

Oxford and Gillespie (1996a,b) demonstrated that the mode of inheritance of the color polymorphism in *T. grallator* was modified subsequent to colonization of the youngest island, Hawaii. The changes are complex, involving a single locus (or linkage group) on Maui but two on Hawaii and two pairs of morphs that are sex-limited on Hawaii (Red Front males/Yellow females, and Red Ring males/Red Blob females) but not on Maui. The genetics of the polymorphism among the different Maui Nui populations seems to be identical (Oxford and Gillespie 1996c) while the situation on Oahu has yet to be explored in detail.

Results from the phylogenetic analyses and migration models using both mtDNA and allozyme data strongly suggest colonization from older to younger islands down the Hawaiian chain and also indicate powerful founder effects reducing variation on newly colonized islands. These analyses support previous conclusions based upon allozymes alone (Gillespie and Oxford 1998) that the Hawaii population was established from Maui, and that current migration between islands is extremely limited. Both the Bayesian phylogenetic and the MIGRATE-N analyses indicated that Mt. Kohala [5] might represent the area where *T. grallator* first colonized Hawaii, with subsequent spread to Kilauea [7] and the Saddle [6]. In the populations of Kilauea [7] (and perhaps also Mt. Kohala [5]), Yellow and Red Front morphs appear to be entirely sex-limited suggesting that this “quantum shift” (Oxford and Gillespie 1996a) in the genetic control of the polymorphism may have originated soon after arrival in Hawaii, probably in the small founder population. The Red Ring and Red Blob sex-limited morphs identified in the Kilauea [7] population are generally rare and their genetics elsewhere on Hawaii has not been established. The congruent signals emerging from these analyses support the argument outlined above, that rare founder events established *T. grallator* populations on successively newer islands with, in one case at least, major upheaval in the genetic architecture of an adaptive trait.

Within the Saddle [6] area, two mechanisms of inheritance for the Yellow and Red Front morphs are present in the same populations (Oxford and Gillespie 1996a). The typical Hawaii pattern of sex-limited morphs predominates but the presence of some Red Front females suggests that a genetic “reversal” of the sex-limitation has occurred in this area. To date, no Yellow males have been identified. That such a reversal may have occurred in the Saddle [6] region rather than elsewhere may not be surprising given the geological activity and shifting-mosaic of fragmented habitat patches (*kipuka*) in this area, leading to repeated founder effects (Carson et al. 1990), a scenario supported by the genetic data, as described above.

The demonstration of a lack of contemporary gene flow among islands, and the colonization of islands through apparently strong founder events, raises an interesting question regard-

ing the nature of the color polymorphism. As mentioned earlier, this comprises a common Yellow morph and a plethora of “patterned” morphs (Oxford and Gillespie 2001) on all islands. Most of the “patterned” morphs are very rare and yet many are found across different islands and populations. Although it is unknown what the diversity of alleles underlying the color morphs would have been at founding, our results suggest that genetic diversity for color may have been reestablished on each island subsequent to the founding event (Oxford and Gillespie 2001). Recent modeling has shown that selection by predators such as leaf-gleaning birds can lead to many different color morphs (Franks and Oxford 2009). Such selection might explain the resurgence of visible genetic diversity in newly established populations that might lack the full complement of color morphs. The reappearance of identical morphs likely results from constraints in pattern development (Oxford 2009). Thus, it appears that the present genetic structure of *T. grallator* populations in Hawaii is best explained by a combination of strong between-island founder events and limited within-island gene flow, leading to stochastic modification of the gene pool, and subsequent selection on the likely depauperate visible variation to recover high levels of genetic polymorphism.

In conclusion, we have focused on the historical biogeography of a single species found on four islands in the Hawaiian archipelago. We have demonstrated a step-like progression down the island chain similar to that shown by suites of closely related species in other lineages (Funk and Wagner 1995), as well as a high level of isolation between populations both between and within volcanoes. Island colonization for *T. grallator* seems to have been a very infrequent event involving few individuals, which fits well with the known quantum shift in genetics underlying the color polymorphism when Hawaii was colonized from Greater Maui (Oxford and Gillespie 1996a). The complexity of population relationships and demographic changes within the dynamic landscape of the youngest island of Hawaii suggests that repeated fragmentation and environmental shifts driven by volcanism and climate change, may have facilitated genetic reshuffling, an argument initially developed by Carson et al. (1990). This study highlights the effects of chance and contingency in evolution, and the interaction of intermittent drift and balancing selection. Drift, presumably “facilitated” the quantum leap in the genetics between Maui and Hawaii, and selection for color variation then built on this to generate the observed (parallel) genetic diversity.

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Data availability: (1) DNA sequences—GenBank Accession Numbers JN863304–JN863390 (see Supporting Information for details); (2) Phylogenetic trees and data matrices—TreeBASE Accession URL: <http://purl.org/phylo/treebase/phylows/study/TB2:S12335>; (3) Allozyme data—Supporting Information.

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Supporting Information

The following supporting information is available for this article:

Figure S1. Final top five migration models and ln Bayes factors (LBF) from Bayesian coalescent model choice (MIGRATE-N).

Figure S2. Fifty percent majority-rule gene-tree constructed from 20,000 posterior trees output by BEAST.

Figure S3. Bayesian skyline plots for individual volcano populations of *T. grallator* and combined plots for island systems (Oahu, Greater Maui, Hawaii) as output by BEAST.

Table S1. Allozyme allele frequencies by *T. grallator* population.

Table S2. Mitochondrial DNA haplotypes, accession numbers, and counts by population.

Table S3. Allozyme genotypes by *T. grallator* population.

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

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