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Influence of volcanic activity on the population genetic structure of Hawaiian *Tetragnatha* spiders: fragmentation, rapid population growth and the potential for accelerated evolution

AMY G. VANDERGAST,* ROSEMARY G. GILLESPIE† and GEORGE K. RODERICK†
*USGS Western Ecological Research Center, San Diego Field Station, 5745 Kearny Villa Road, Suite M, San Diego CA 92123, USA, †Department of Environmental Science, Policy and Management, Division of Insect Biology, University of California, Berkeley, 201 Wellman Hall #3112, Berkeley, CA 94720–3112, USA

Abstract

Volcanic activity on the island of Hawaii results in a cyclical pattern of habitat destruction and fragmentation by lava, followed by habitat regeneration on newly formed substrates. While this pattern has been hypothesized to promote the diversification of Hawaiian lineages, there have been few attempts to link geological processes to measurable changes in population structure. We investigated the genetic structure of three species of Hawaiian spiders in forests fragmented by a 150-year-old lava flow on Mauna Loa Volcano, island of Hawaii: *Tetragnatha quasimodo* (forest and lava flow generalist), *T. anuenue* and *T. brevignatha* (forest specialists). To estimate fragmentation effects on population subdivision in each species, we examined variation in mitochondrial and nuclear genomes (DNA sequences and allozymes, respectively). Population subdivision was higher for forest specialists than for the generalist in fragments separated by lava. Patterns of mtDNA sequence evolution also revealed that forest specialists have undergone rapid expansion, while the generalist has experienced more gradual population growth. Results confirm that patterns of neutral genetic variation reflect patterns of volcanic activity in some *Tetragnatha* species. Our study further suggests that population subdivision and expansion can occur across small spatial and temporal scales, which may facilitate the rapid spread of new character states, leading to speciation as hypothesized by H. L. Carson 30 years ago.

Keywords: genetic subdivision, habitat fragmentation, Hawaii, population growth

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Introduction

The Hawaiian archipelago is renowned for some of the most remarkable species radiations in the world, including the drepanid honeycreepers (Tarr & Fleischer 1995), the silversword alliance of composite plants (Baldwin 1997; Baldwin & Wessa 2000), picture-winged *Drosophila* flies (Carson & Kaneshiro 1976), land snails (Thacker & Hadfield 2000; Holland & Hadfield 2002), *Laupala* crickets (Shaw 1996; Shaw & Herlihy 2000) and *Tetragnatha* spiders (Gillespie *et al*. 1994; Gillespie 2004). Therefore, it is no surprise that the biogeographic patterns and mechanisms underlying speciation in Hawaiian organisms have long been of great interest to evolutionary researchers.

Overwhelmingly, phylogeographic studies have reported that patterns of Hawaiian species radiations mirror the geological formation of the Hawaiian island chain (Wagner & Funk 1995; Shaw 1996; Roderick & Gillespie 1998; Price & Clague 2002; Gillespie 2004). Geologic evidence suggests that the Hawaiian Islands were formed successively over a fixed 'hot spot' beneath the northwestward moving Pacific tectonic plate (Wilson 1963). This hotspot sits currently at the southern tip of the youngest island, the Big Island of Hawaii. Moving southward along the island chain one encounters increasingly younger islands, and for many Hawaiian lineages, more derived species (Funk & Wagner 1995). Additionally, many Hawaiian species

Correspondence: Amy G. Vandergast. Fax (858) 974 3563; E-mail: avandergast@usgs.gov

complexes contain further intra-island radiations, usually with single-volcano endemics (Carson & Clague 1995). If pattern reflects process, then the link between the diversity of Hawaiian taxa and the geology of the islands suggests that geologic processes may be important factors promoting diversification and speciation within Hawaiian lineages (Carson & Templeton 1984; Carson 1990; Roderick & Gillespie 1998). On the Big Island of Hawaii, these dynamic geologic processes are still occurring. Ongoing volcanic activity has created new land at the southern tip of the island, and the southward pattern of older to younger volcanoes mirrors the formation of the island chain on a smaller scale. On the youngest and most active of these Big Island volcanoes, Mauna Loa and Kilauea, lava flows bury surfaces at rates of about 40% and 90% per 1000 years, respectively (Carson *et al*. 1990). Ongoing volcanic activity has created a shifting mosaic of habitats as large areas of forest are destroyed or fragmented into small habitat 'islands' (called *kipuka*) when lava flows over or around them. After a lava flow cools the forest gradually regenerates, receiving founders from adjacent intact areas. A mature, closed canopy forest has been estimated to develop in 300–3000 years on new flows, with temporal differences depending on abiotic conditions (e.g. slope, aspect, altitude and lava type) and biotic factors (e.g. presence of invasive species; Kitayama *et al*. 1995; Clarkson 1998). Consequently, populations of forest-dwelling organisms on the slopes of these volcanoes have been subject to repetitive extinctions, fragmentation, founder events and subsequent population growth. Based on studies of *Drosophila*, Carson and colleagues hypothesized that a combination of metapopulation structure and founder effects resulting from the geologic activity on Hawaiian shield volcanoes has been important in creating and maintaining genetic differences among populations and, ultimately, in the evolution of new character states and species (Carson & Sato 1969; Carson & Templeton 1984; Carson 1990; Carson *et al*. 1990). Indeed, the isolation of small populations due to lava flows has been invoked in explanations of phylogenetic patterns in several Hawaiian lineages (Thornton 1984; Gillespie & Croom 1995; Givnish *et al*. 1995; DeMeyer 1996). However, with the exception of the Hawaiian *Drosophila* (Carson & Johnson 1975; Carson *et al*. 1990), no endemic Hawaiian taxa have been studied at the population level across recent lava flows in order to determine the influence of volcanic activity on population genetic structure.

Using information from mitochondrial DNA sequences and nuclear allozyme loci, we investigated the population genetic structure of three closely related species of Hawaiian *Tetragnatha* (Araneae Tetragnathidae) in small forest *kipuka* isolated by an 1855 lava flow on the eastern slopes of Mauna Loa. The primary goal of this study was to determine whether this recent fragmentation event has led to increased population subdivision. Additionally, we used the genealogical information contained within mtDNA sequences to reconstruct the demographic history of these species, with the expectation that prior habitat destruction and subsequent regeneration on new substrates would leave a signature of population expansions following bottlenecks. To our knowledge, this is the first study to test explicitly the hypothesis that geologic processes on active volcanoes will alter population genetic structure and demography and thus, potentially influence the evolutionary trajectories of Hawaiian arthropods.

Methods

Hawaiian Tetragnatha

The genus *Tetragnatha*, the long-jawed orb-weaving spiders, is of worldwide distribution, associated generally with riparian and littoral habitats. The genus is known for its remarkable dispersal abilities by means of ballooning, comprising almost all of the aerial spider plankton collected offshore in the China Sea (Okuma & Kisimoto 1981). Therefore, perhaps not surprisingly, the genus has colonized the Hawaiian Islands more than once (Gillespie *et al*. 1994). However, within the Hawaiian Islands, the genus has undergone a substantial species radiation (Simon 1900; Gillespie 1991; Gillespie 1992; Gillespie 1994; Gillespie 2002) and, in common with many Hawaiian taxa, dispersal abilities are thought to be much reduced: all native *Tetragnatha* are endemic, most have very limited ranges within the islands, and there is no direct evidence of dispersal by ballooning. Molecular data suggest that *Tetragnatha* species have 'hopped down' the island chain from older to younger islands. In addition, some species tend to be related more closely within an island than between islands, suggesting that speciation has occurred repeatedly within a single island, most probably through shifts in microhabitat and/or prey specialization (Gillespie *et al*. 1997). Hawaiian *Tetragnatha* vary in their habitat use, with some species widely distributed across habitat types and others highly restricted (Gillespie 1997). Three species that co-occur in the *kipuka* system of Hawaii Island were chosen for study: *T. brevignatha* (Gillespie 1991), *T. anuenue* (Gillespie 2002) and *T. quasimodo* (Gillespie 1991). These three species belong to the 'spiny-leg clade' of Hawaiian *Tetragnatha*, in which all members have abandoned web-building (Gillespie *et al*. 1997). In ecological surveys conducted across forest edges in the *kipuka* system, these three species were the only representatives of the spiny-leg clade encountered (Vandergast 2002). *T. brevignatha* and *T. anuenue* were restricted to the interiors of remaining forest fragments, while *T. quasimodo* resided both within forest fragments and on the sparse vegetation of the younger surrounding lava flows (Vandergast 2002). This ecological difference led us to predict that forest-restricted species (*T. brevignatha*

and *T. anuenue*) could suffer reduced gene flow if dispersal is inhibited by habitat fragmentation, while the generalist species (*T. quasimodo*) would not.

Although ballooning cannot be ruled out as a dispersal mechanism in these three species, it is unlikely to be important for forest specialists in the fragmented *kipuka* landscape. The understorey vegetation of forested patches is dense, especially at fragment edges. Ballooning individuals are more likely to be snagged on branches and remain within the forest *kipuka* than to be dispersed to adjacent patches. Bonte *et al*. (2003) found similarly that ballooning performance in spiders is related negatively to habitat specialization in fragmented habitats. Cursorial Hawaiian *Tetragnatha* are also known to run actively along the vegetation (Gillespie *et al*. 1997), which is probably an important dispersal mechanism in areas of contiguous suitable habitat. For the two forest specialists, suitable habitat is limited to forest understorey. However, *T. quasimodo* also is found in the much sparser vegetation on surrounding lava flows (Vandergast 2002). Thus, we predicted that dispersal (and hence, gene flow) would be curtailed in forest specialists in this fragmented system. Genetic studies have repeatedly shown that differences in gene flow estimates among species reflect actual dispersal differences when the species are closely related, and the most important determinants of dispersal are known (reviewed by Bohonak 1999). Therefore, the three *Tetragnatha* species chosen represent an appropriate model for investigating the genetic effects of habitat fragmentation.

Study site

The *kipuka* system investigated consists of mesic forest fragments surrounded by an 1855 lava flow originating from Mauna Loa Volcano (Fig. 1; general map coordinates: N 19°37′40″ and W 155°21′15″). We focused specifically on seven small forest *kipuka* and five collection points within a continuously forested area of a much larger *kipuka*. These *kipuka* range in age from approximately 750–1500 years BP (based on radiocarbon dating of lava substrates), and were most probably connected prior to the 1855 flow that currently surrounds them (Lockwood *et al*. 1988). Field surveys showed that the smaller *kipuka* interiors were similar to the continuous forest interior in terms of climate, vegetation and spider species composition (Vandergast 2002). Edge effects are minimal, affecting vegetation structure only 10–20 m into forest interiors. Therefore, fragments are very likely to contain small populations that persisted during the fragmentation event, rather than being recolonized following local extinctions. We also assumed that levels of genetic differentiation among collection points in the continuous forest would be similar to that of currently fragmented populations *before* this fragmentation event occurred.

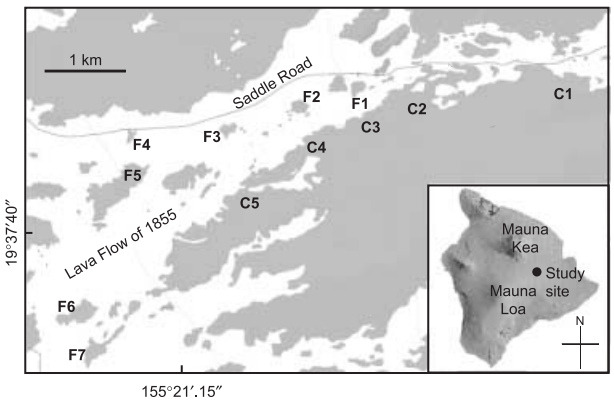

Fig. 1 Map of the study site on the Saddle Road, Island of Hawaii. The general location of the study site is marked on the inset drawing of the Island of Hawaii. Forested areas are dark grey and are surrounded by an 1855 lava flow from Mauna Loa Volcano. Sites F1–F7 are small forest fragments; sites C1–C5 are located in a large stretch of continuous forest. Sample sizes for mtDNA and allozymes, respectively, are as follows: *T. anuenue*: F1 (15, 23); F2 (27, 19); F3 (13, 38); F4 (17, 57); F5 (10, 20); F6 (10, 14); F7 (8, 17); C1 (0, 0); C2 (18, 17); C3 (14, 18); C4 (12, 22); C5 (14, 31). *T. brevignatha*: F1 (14, 17); F2 (16, 14); F3 (11, 15); F4 (0, 0); F5 (6, 7); F6 (0, 0); F7 (0, 0); C1 (8, 7), C2 (15, 17); C3 (12, 21); C4 (13, 20); C5 (16, 19). *T. quasimodo*: F1 (9, 21); F2 (6, 23); F3 (10, 19); F4 (10, 33); F5 (11, 19); F6 (9, 13); F7 (9, 12); C1 (6, 6); C2 (9, 16); C3 (9, 16); C4 (10, 19); C5 (8, 4).

Collections

Spiders were collected during April and May 1997–2000 at each of the seven isolated forest fragments (F1–F7; Fig. 1) and from five areas within the continuously forested area of the large *kipuka* (C1–C5; Fig. 1). All species were collected from each study site with the following exceptions: *T. brevignatha* was not found in fragments F6 and F7 and *T. anuenue* was not found in C1, despite intense collecting effort in these locations. Collected spiders were brought back to the laboratory and stored at −80 °C.

Amplification and sequencing of mtDNA

Sequences were collected from six to 27 individuals per population of each species (see Fig. 1 caption). Genomic DNA was isolated from one to three legs of individual spiders using DNEASY Tissue Kits (Qiagen, Valencia, CA, USA). For *T. brevignatha* and *T. anuenue* samples, a 708 base pairs (bp) region of the mitochondrial cytochrome oxidase I (COI) gene was amplified using the universal primer pair Lco1490: 5′-GGTCAACAAATCATAAAGATATTGG and Hco2198: 5′-TAAACTTCAGGGTGACCAAAAAATCA (Folmer *et al*. 1994). For *T. quasimodo* samples, we used an alternate primer pair (LCO1628: 5′-ATAATGTAATTGTT-ACTGCTCATGC and HCO2396: 5′-ATTGTAGCTGAG-GTAAAATAAGCTCG), designed in the Roderick and Gillespie laboratories, to amplify a 768 bp region of the

COI gene. The two primer pairs amplified an overlapping region of 570 bp. Using a thermal cycler, amplifications were as follows: 95 °C for 2 min; 35 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 40 s; 72 °C for 7 min. Amplification reactions consisted of 2 μL of DNA, 0.5 U Ampli*Taq* DNA polymerase (Applied Biosystems), 1.8 mM $MgCl_2$, 0.2 mM each dNTP and 0.4 mM each primer in 25 μL total volume. Polymerase chain reaction (PCR) products were purified using the QIAquick PCR Purification Kit (Qiagen). PCR products were sequenced in both directions using Big Dye Terminator (Applied Biosystems) and run on an ABI 377 automated sequencer. Resulting sequences were aligned manually in SEQUENCHER (version 3.1.1; Gene Codes Corporation). No insertions or deletions were found, and ambiguous end regions were removed so that all individuals within each species were analysed over the same sequence length. After alignment and cropping, a 611 bp segment was analysed for *T. quasimodo*, 607 bp for *T. anuenue* and 605 bp for *T. brevignatha*. Unique haplotypes were determined using the program COLLAPSE version 1.1 (Posada 1999).

Allozymes

Based on an initial screening of 21 enzyme systems, acetate gel electrophoresis was used to score presumptive polymorphic loci at eight enzyme systems from samples taken from the same locations that COI sequences were obtained (Fig. 1 caption). These were glyceraldehyde-3-phosphate dehydrogenase (G3PDH, EC 1.2.1.12), 6-phosphogluconate dehydrogenase (6PGDH, EC 1.1.1.44), glucose-6-phosphate isomerase (PGI, EC 5.3.1.9), phosphoglucomutase (PGM, EC 5.4.2.2), aspartate amino transferase (AAT, EC 2.6.1.1), adenylate kinase (APK, EC 2.7.4.3), malate dehydrogenase (MDH, EC 1.1.1.37) and isocitrate dehydrogenase (IDH, EC 1.1.1.42). Gels were run at 180 V for between 35 and 60 min (depending on the enzyme system) at 4 °C, and stained to visualize protein products following published protocols (Hebert & Beaton 1993). Allozyme loci were scored separately for each species and population allele frequencies were calculated.

Analysis

Knowledge of the geologic history of the study site and ecological differences among the three spiders led to three hypotheses concerning past demographic changes and present population structure. First, recent fragmentation by lava will lead to increased population genetic structure among isolated fragments, when compared to populations in continuous forest. Second, populations of forest-restricted specialists are more divergent across recent lava flows than the generalist species, which is found nearly everywhere. Finally, prior episodes of fragmentation followed by subsequent habitat regeneration will leave a genetic signature of population expansion following bottlenecks.

Does lava flow prevent gene flow?

The first hypothesis was tested with three approaches. First, exact χ^2 tests for population differentiation were performed for all genetic markers (Raymond & Rousset 1995) and as suggested by Excoffier *et al*. (1992), the null hypothesis of no mtDNA population structure was tested using 10 000 permutations of the AMOVA test statistic in ARLEQUIN version 2.0 (Schneider *et al*. 2000). Second, within each species, we compared 95% confidence intervals for the genetic differentiation statistic θ among populations separated by lava (F1–F7) to θ among forest collection points (C1–C5). Weir & Cockerham's (1984) θ, an estimate of Wright's F_{ST}, was calculated for allozymes over all polymorphic loci with the program TFPGA (Miller 1993). Estimates of θ based on mtDNA were determined in ARLEQUIN. For allozymes, confidence intervals (95%) were obtained by 10 000 bootstrap replications over loci. Because mtDNA sequences constitute a single linked locus, 95% confidence intervals for θ from mtDNA were obtained by jackknifing over populations (following Weir 1990).

Finally, the first hypothesis was tested using analyses of 'isolation by distance' (Slatkin 1993). For both marker sets, pairwise linearized F_{ST} (Rousset 1997) was estimated between all population pairs and plotted against the log-transformed geographic distance. Significant correlations between genetic and geographic distance matrices were determined with Mantel tests. To examine the effects of lava on genetic structure, we also created a binary 'fragmentation matrix' with values of 1 for population pairs separated by lava and 0 for those separated by forest. Partial Mantel tests (Legendre & Legendre 1983) were then employed to assess the correlation between genetic distance and geographic distance after controlling for fragmentation and, conversely, the correlation of genetic distance with fragmentation while controlling for geographic distance. 'Isolation by distance' analyses were performed in the program IBD version 1.5 (Bohonak 2002).

Population subdivision for mtDNA is often quantified using statistics such as Φ_{ST} (Excoffier *et al*. 1992), which take into account the number of mutations between alleles, or analysed with genealogically based methods such as nested clade analyses (NCA) (Templeton 1998). We chose not to use these methods because in less than 150 years following fragmentation, new mutations are unlikely to have reached detectable frequencies. Thus, genealogical relationships among haplotypes will reflect population structure prior to the fragmentation event, rather than after. Haplotype relatedness will not become informative until new alleles have appeared in each *kipuka* after a much longer time. Consistent with this, preliminary sets of NCA found

no significant geographic associations concordant with fragmentation for any clade in any species (Vandergast 2002).

Does fragmentation affect forest specialists more than the generalist?

To test the second hypothesis, we compared the 95% confidence intervals for θ among the three species for both types of genetic markers.

Have population expansions left distinct genetic signatures?

Because the fragmentation event was recent, we expected that genealogical relationships among mtDNA haplotypes would reflect population processes prior to the lava flow of 1855. Information about a particular species' history can be inferred from its genealogical structure. Past population growth, for example, can be inferred if the most basal branches of the sampled genealogy are relatively short compared to those expected in a population of stable size (Kuhner *et al*. 1998). Because preliminary mtDNA NCA suggested that *kipuka* populations were panmictic prior to the most recent fragmentation event (see above), all sampled sequences from each species were treated as a single 'population' for tests of the third hypothesis.

We utilized two methods to explore the demographic history of each species. First, mutation-scaled effective population sizes ($\theta = N_e\mu$) and population growth rates (g) were estimated from sampled sequences following a maximum likelihood search algorithm in the program FLUCTUATE version 1.4 (Kuhner *et al*. 1998). Second, we adapted the skyline plot method of Strimmer & Pybus (2001) to investigate the shape of the population growth curve through time. These estimates were derived in two independent steps: (1) phylogenetic estimation of gene trees under the assumption of a molecular clock and (2) coalescent estimation of the change in population size through time based on the phylogenetic trees obtained. Sets of phylogenetic trees were generated using a Bayesian search method. Searches were performed in MR BAYES 2.0 (Huelsenbeck & Ronquist 2001) using a GTR + I model (Rodríguez *et al*. 1990), and enforcing a molecular clock. Each search started from a random tree and ran for 2 million generations, sampling every 1000 th generation. The first 200 cycles (10%) were discarded as burn in, and the remaining 1800 trees retained. Searches were repeated two additional times to confirm stationarity. From the resulting 5400 trees in the stationary distribution, 100 trees were selected randomly for demographic analysis (tree files available upon request). Demographic history was visualized using an adaptation of generalized skyline plot method (Strimmer & Pybus 2001), which uses the coalescent to estimate $N_e\mu$ backwards through time (measured in nucleotide substitutions per site). The method results in an estimate of the demographic history of a population based on the branching pattern and depth of the underlying genealogy. Generalized skyline plots were estimated in the program GENIE 3.0 (Pybus & Rambaut 2002) and were replicated over each of the 100 randomly selected trees. For each time step we calculated the mean and 95% confidence interval around $N_e\mu$ and these were plotted against the number of substitutions per site. Plots were then compared visually among species.

Results

COI sequence variation

Forty-five unique haplotypes were detected in *T. quasimodo*, 30 haplotypes were found in *T. anuenue* and 27 haplotypes were found in *T. brevignatha*. The number of polymorphic sites, the number of transistions and transversions and the average pairwise distance among haplotypes are presented for each species in Table 1. All haplotype sequences were submitted to GenBank under the following Accession nos: AY530430–AY530531. The relationships among haplotypes within each species are displayed as networks in Fig. 2.

Allozyme variability

In total, eight polymorphic loci were resolved for *T. quasimodo* (PGI, PGM, AAT, APKs, IDHs, IDHf, MDH and G3PDH) with a range of two to four alleles per locus. Six polymorphic loci were resolved for *T. anuenue* (PGI, PGM, AAT, IDHs, IDHf, 6PGDH) with between two and seven alleles per locus. Seven polymorphic loci were resolved for *T. brevignatha* (PGI, PGM, AAT, IDHs, IDHf, MDH, 6PGDH) with between two and four alleles per locus.

Table 1 mtDNA COI sequence variation for the three species examined

Species	*n*	No. of haplotypes	No. of nucleotide sites	No. of polymorphic sites	No. of transitions	No. of transversions	Mean sequence distance
T. anuenue	157	30	607	29	29	1	1.0%
T. brevignatha	111	27	605	34	29	6	0.8%
T. quasimodo	129	45	611	58	50	11	1.5%

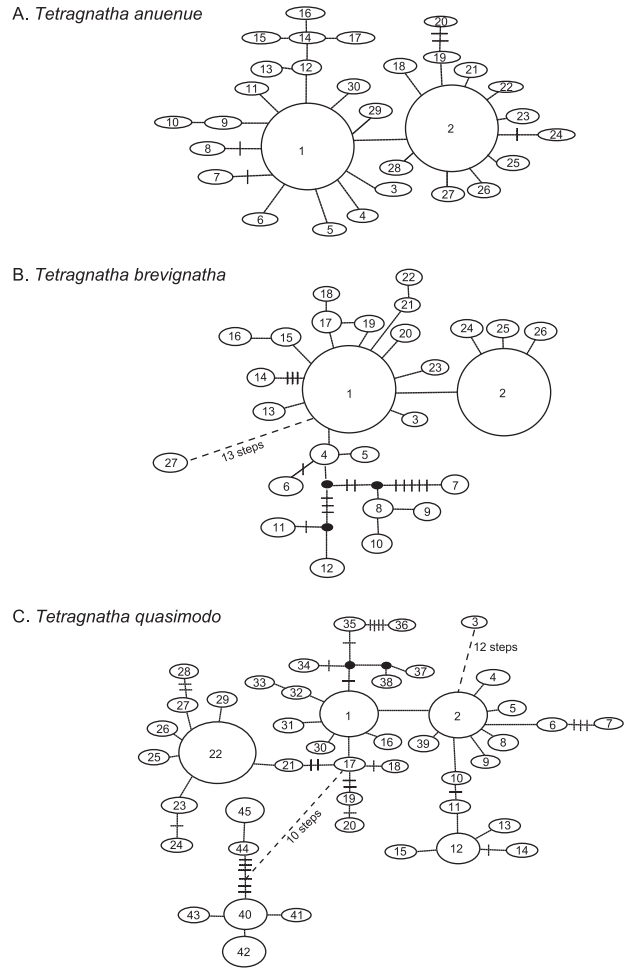

Fig. 2 Haplotype networks for all three species. Networks were created using a maximum parsimony algorithm in the program TCS version 1.13 (Clement *et al*. 2000). Sampled haplotypes are numbered and missing haplotypes are shown as black dots and slashes. Haplotypes and clades connected with dashed lines could not be connected with 95% probability in TCS, and are connected to their most closely related haplotypes based on a matrix of absolute pairwise differences. Larger circles represent more common haplotypes.

No departures from Hardy–Weinberg equilibrium were detected at any locus for any population. Allele frequencies for each locus and population are provided in Appendices I–III.

Hypotheses

Each of the three hypotheses regarding current population structure and past demographic history was supported by one or more statistical tests. Results are described in detail below and summarized in Table 2.

(1) Does lava flow prevent gene flow? For mtDNA sequences, statistically significant population structure was measured among populations separated by lava for both *T. anuenue* and *T. brevignatha* (AMOVA; Table 3). Exact tests for population differentiation supported this result for allozyme loci as well. In contrast, among populations separated by forest, both forest specialists showed very low estimates of θ that were not significantly different from zero in either mtDNA or allozymes. In *T. anuenu*e, 95% confidence intervals around θ derived from mtDNA did not overlap, supporting significantly greater population differentiation in lava populations than in forest populations (Table 3), but differences were not significant for allozymes (see 'Interpreting F_{ST} in nonequilibrium conditions' below).

A similar examination of confidence intervals around θ in *T. brevignatha* did not differentiate between lava and forest populations in either marker; however, differences between these habitat types were revealed in the isolation by distance analysis. In pairwise comparisons based on mtDNA and allozymes of *T. brevignatha*, we found a significant correlation between genetic and geographic distance when corrected for fragmentation, and a significant effect of fragmentation when corrected for geographic distance (Fig. 3, Table 4). In contrast, genetic and geographic distance were not related statistically for forest or lava populations of *T. anuenue* and *T. quasimodo* for either marker (Fig. 3, Table 4).

Table 2 Evidence supporting three major hypotheses

Hypothesis	Supporting evidence
1. Greater population structure in fragmented vs. nonfragmented conditions	T. *anuenue*: mtDNA; non-overlapping confidence intervals around estimate of θ *T. brevignatha*: mtDNA and allozymes; significant partial correlation of genetic distance and fragmentation index *T. quasimodo*: no evidence of population structure
2. Greater population structure in habitat specialists vs. generalist	*T. anuenue*: mtDNA; non-overlapping confidence intervals around θ in fragmented populations when compared to *T. quasimodo* *T. brevignatha*: no statistical support
3. Signature of past population expansion	All species: positive growth rates obtained with maximum likelihood estimates and skyline plots

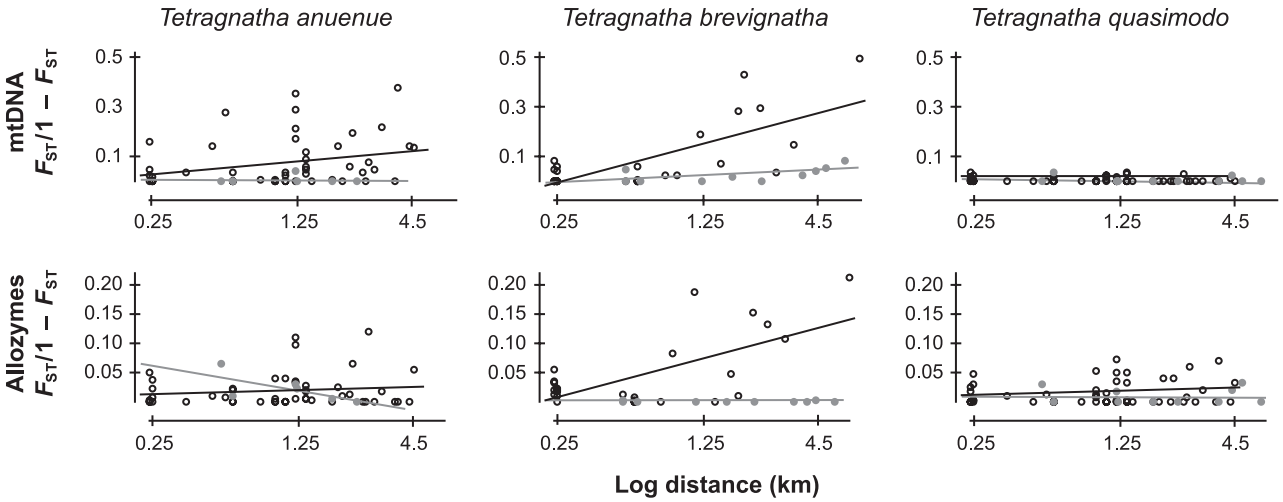

Fig. 3 Plots of pairwise linearized F_{ST} ($F_{ST}/1 - F_{ST}$; based on mtDNA or allozymes) by geographic distance for all species. Geographic distance is log-transformed. Open black circles represent population pairs separated by lava and population pairs separated by forest are in closed grey circles.

Table 3 Summary of *F*-statistics (estimated as θ; Weir & Cockerham 1984) for all species for populations separated by forest and lava, respectively. For mtDNA sequences, significance assessed from 10 000 permutations of the AMOVA test statistics as implemented in ARLEQUIN and 95% confidence intervals were obtained by jackknifing over populations (Weir 1990). For allozymes, 95% confidence intervals were obtained by bootstrapping (10 000 replicates) over loci

	T. anuenue		*T. brevignatha*		*T. quasimodo*	
F_{ST}	Forest	Lava	Forest	Lava	Forest	Lava
θ mtDNA	−0.0148	0.0817*†	0.0011	0.0731*	−0.0221	−0.0151
95% CI	(−0.0534, 0.0311)	(0.0496, 0.1174)	(−0.0270, 0.0085)	(−0.0554, 0.1495)	(−0.0444, 0.0027)	(−0.0232, −0.0047)
θ Allozymes	0.0196	0.0112†	−0.0097	0.0142†	0.0007	0.0046
95% CI	(−0.0024, 0.0488)	(−0.0053, 0.0298)	(−0.0164, 0.0075)	(−0.019, 0.032)	(−0.0156, 0.0305)	(−0.0077, 0.0288)

*Significantly greater than zero based on AMOVA permutations ($P < 0.05$).
†Significant population differentiation based on exact χ^2 tests ($P \leq 0.05$).

(2) Does fragmentation affect forest specialists more than the generalist? In both forest specialist species, significant population structure was measured in populations separated by lava. Conversely, fragmented populations of the generalist *T. quasimodo* were not differentiated. In *T. anuenue*, the 95% confidence intervals around θ derived from mtDNA for lava populations did not overlap with those estimated for *T. quasimodo*, lending limited support to this hypothesis (Table 3). However, there is no indication of greater population structure in *T. brevignatha* lava populations when compared to *T. quasimodo*, based on confidence intervals alone. Overall, these patterns suggest that *T. anuenue* and *T. brevignatha* have experienced some level of population subdivision due to habitat fragmentation, while populations of *T. quasimodo* appear to have remained panmictic.

(3) Have population expansions left distinct genetic signatures? Maximum likelihood estimates of population growth obtained from FLUCTUATE were positive in all species (Table 5). Skyline plots corroborated these results (Fig. 4). All three species appear to have undergone past population growth, with some apparent differences in the timing of that growth. Based on skyline plots with steeper slopes originating more recently in the past, *T. anuenue* and *T. brevignatha* appear to have undergone fairly recent and rapid population expansions (Fig. 4A,B). In contrast, population growth in *Tetragnatha quasimodo* has been more moderate through time, as evident by a more gradual slope (Fig. 4C).

Discussion

Population subdivision as a result of habitat fragmentation

Species with high levels of population differentiation among forest fragments could be restricted as a result of

Table 4 Results from Mantel tests for matrix correlation between genetic and geographic distances, and partial Mantel tests for matrix correlation between genetic, geographic distance and a fragmentation indicator matrix (IBD version 1.6; Bohonak 2002). Significance assessed with 20 000 randomizations of the genetic matrix

	T. anuenue		*T. brevignatha*		*T. quasimodo*	
Correlation coefficients	mtDNA	Allozymes	mtDNA	Allozymes	mtDNA	Allozymes
Genetics and distance	0.2587†	0.0825	0.5218*	0.3269	−0.1027	0.0572
Genetics and distance (controlled for fragmentation)	0.2731†	0.0813	0.6931*	0.5717*	−0.1172	0.0784
Genetics and fragmentation (controlled for distance)	0.2365†	−0.0348	0.5646*	0.5885*	−0.0651	0.0679

*$P \leq 0.05$.
†$0.10 < P < 0.05$.

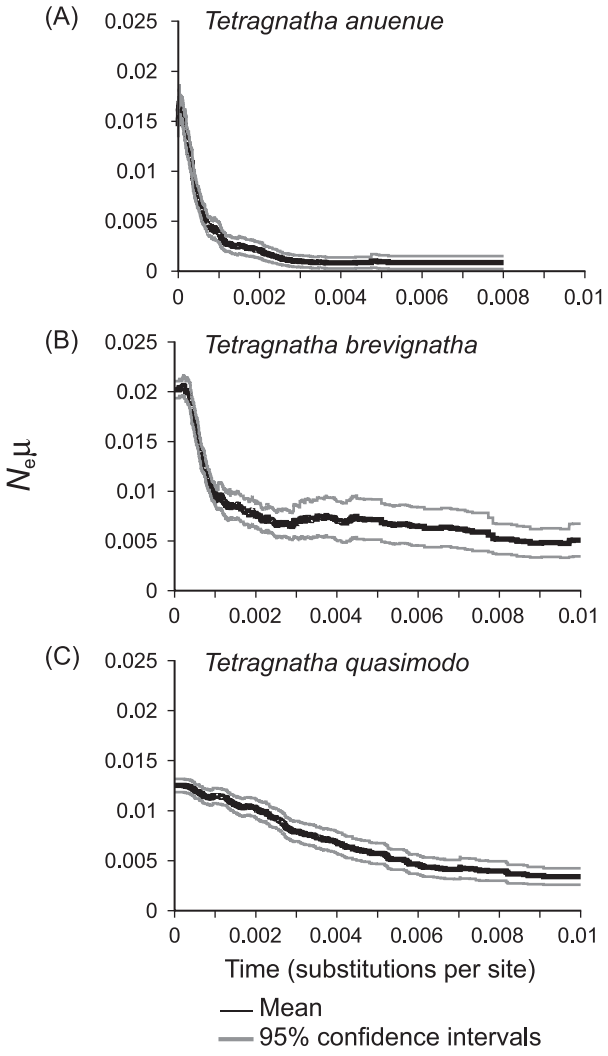

Fig. 4 Skyline plots for *T. anuenue* (A), *T. brevignatha* (B) and *T. quasimodo* (C). Black lines represent the mean population growth estimates of 100 skyline plots based on randomly selected genealogical estimates, moving backwards through time; 95% confidence intervals around the mean are shown in grey. Plot shapes suggest recent and sudden growth in *T. anuenue* and *T. brevignatha* and slower, steadier growth in *T. quasimodo*.

Table 5 Simultaneous maximum likelihood estimates and standard deviations for nucleotide diversity ($\theta = N_e\mu$, assuming $Nm = Nf$) and population growth rate (g) of each species as estimated in the program FLUCTUATE. Starting parameters for each analysis used empirical base frequencies, empirical transistion/transversion ratios, Watterson's (1975) estimate of θ and $g = 1$

	T. anuenue	*T. brevignatha*	*T. quasimodo*
$N_e\mu$	0.1854 (0.0115)	0.0207 (0.0037)	0.0674 (0.0044)
g	2420.1566 (76.9747)	320.9441 (91.1077)	200.4379 (25.1479)

fragmentation, or they may have naturally patchy and restricted distributions even in nonfragmented conditions. By comparing levels of genetic differentiation among fragments to those across similar distances of continuously forested habitat, we have presented evidence that habitat fragmentation is the probable causal factor increasing population subdivision in *T. anuenue* and *T. brevignatha*. For genetic markers representing mitochondrial and nuclear genomes, significant divergence was measured among populations separated by lava but not among adjacent populations separated by forest. More importantly, lava populations of *T. anuenue* were significantly more differentiated than forest populations for mtDNA. Finally, in both sets of markers, *T. brevignatha* showed evidence of increasing genetic isolation with distance for populations separated by lava; however, in populations separated by forest, genetic differentiation was negligible regardless of distance. These differences are even more striking when compared to the habitat generalist, *T. quasimodo*, in which no population subdivision was detected across habitat groupings for either marker.

Ecological studies of species distributions under fragmented conditions have shown generally that species with varying habitat requirements and life history traits respond differently to habitat fragmentation (Fahrig & Grez 1996; Didham *et al*. 1998; Kolozsvary & Swihart 1999). Species with small populations and specialized habitat needs tend

to decline while generalist and opportunistic species may be unaffected or thrive (Lynam 1997; Gascon *et al*. 1999; Bolger *et al*. 2000). This difference may be caused by a greater reduction in suitable habitat for specialists and a greater reduction in connectivity among populations if specialists cannot disperse through the surrounding habitat. Our genetic results in the *kipuka* system support this latter point; fragmentation disrupted the genetic continuity of forest specialists, but did not affect the generalist.

Interpreting F_{ST} *in nonequilibrium conditions*

In both *T. anuenue* and *T. brevignatha*, results from mtDNA sequences are more indicative of a fragmentation effect than results based on allozyme loci. We interpret the mismatch between mitochondrial and nuclear markers to indicate that populations of spiders in isolated forest *kipuka* have yet to reach a genetic equilibrium between drift and gene flow (Bohonak & Roderick 2001). With an effective population size one-quarter that of nuclear genes, mtDNA is expected to approach equilibrium more quickly, explaining the higher and more significant estimates of F_{ST} derived from mtDNA. An alternative explanation for this pattern is that male *T. anuenue* and *T. brevignatha* may be moving among fragments more frequently than females. In detailed observations of these and related cursorial tetragnathids, there has been no indication of sex-biased movement or dispersal. Thus, nonequilibrium conditions provide a more probable explanation. Consequently, it may be misleading to interpret F_{ST} quantitatively in terms of the number of migrants per generation (Bossart & Prowell 1998; Whitlock & McCauley 1999). Nonetheless, the qualitatively different patterns found in fragmented vs. nonfragmented habitats and between specialist and generalist species provide evidence that fragmentation has altered the genetic structure of the two forest specialists.

Evidence for population expansion

Estimates of population growth and skyline plots indicated that all three species have experienced recent expansions. The contrasting shapes of the skyline plot growth curves suggest further that population expansion has been more recent and rapid in the two forest specialists than in the habitat generalist. These signatures of population expansion may reflect historical processes in *kipuka* populations. Our study site on the eastern slope of Mauna Loa has been subject to a continuous cycle of habitat destruction and fragmentation by lava flows and subsequent forest regrowth (Lockwood *et al*. 1988). Ecological studies of ecosystem development on these eastern Mauna Loa flows have estimated that a mature closed-canopy forest with a tree fern understorey (necessary to support populations of *T. anuenue* and *T. brevignatha*) may take between several hundred to several thousand years to develop on barren flows (Kitayama *et al*. 1995; Aplet *et al*. 1998). It is likely that past lava flows have led to fragmented populations such as those seen now, followed by population expansion as individuals recolonize areas where forests have regenerated. The more stable pattern of population growth in *T. quasimodo* is expected, given its ability to survive in a wider range of habitat substrates, which allows this species to colonize much younger flows. It is clear that volcanic processes on this island have left the distinct genetic signature of population expansion in these lineages.

Potential links to speciation in Hawaiian Tetragnatha

This study demonstrated that geologic processes on active volcanoes have altered the population genetic structure and demography of some Hawaiian tetragnathids. Populations appear to have grown in size, and in two species, genetic subdivision has increased due to the most recent lava flow. These genetic signatures are concordant with the geologic history of lava flow, forest fragmentation and regeneration on Mauna Loa Volcano. Although these demographic fluctuations are similar to the conditions under which founder-flush speciation models have been hypothesized to act (Carson & Templeton 1984), whether or not this cycle facilitates the spread of new character states or traits under selection remains to be determined for *Tetragnatha*. Theoretical studies provide some insight to the potential influence of such demographic changes. For example, in small populations, random genetic drift is the predominant evolutionary force, overwhelming weak levels of selection (Slatkin 1996). Conversely, simulations comparing expanding and stable populations suggest that rapid population growth greatly increases the role that selection may play (Slatkin 1996; Otto & Whitlock 1997). Consequently, cyclic patterns of population contraction and growth could facilitate the fixation of beneficial character states that may have initially been present in very low frequencies (Slatkin 1996). While such an event has yet to be documented in Hawaiian *Tetragnatha*, current phylogenetic and ecological data do suggest that adaptive shifts in microhabitat and prey specialization have been important factors leading to rapid speciation in the group (Gillespie *et al*. 1997; Oxford & Gillespie 2001). On each of the older islands (all except Hawaii), species within the spiny leg clade have undergone extensive within-island diversification, associated with adaptive shifts and the adoption of specific ecological roles (Gillespie 2004). The age of the young island of Hawaii may be insufficient to have allowed speciation through adaptive shifts. Most species on Hawaii have colonized from the next older island, Maui. However, the patterns revealed in this study provide demographic insight into how diversification through adaptive shifts may be initiated in this group.

Interestingly, *T. quasimodo* is the only species in the spiny leg clade that is very widespread, being found on all islands from Oahu to Hawaii. The lack of diversification within this species may be the result of its generalist lifestyle and lack of population subdivision.

Conclusions

Carson and colleagues first hypothesized that patterns of lava flows could be important in the speciation process of Hawaiian lineages nearly 30 years ago (Carson & Johnson 1975). Studies of Hawaiian *Drosophila* confirmed that population-level changes occurred across even small geographical scales due to fragmentation and founder events across flows of varying ages (Carson *et al*. 1990). Despite the ideological debate that ensued (Barton & Charlesworth 1984; Carson & Templeton 1984; Charlesworth 1997), there have been few, if any, attempts to gather empirical support for Carson's theory in other Hawaiian taxa. We have presented evidence that populations of certain Hawaiian *Tetragnatha* species are subject to increased genetic subdivision due to fragmentation by lava, and contain a genetic signature concordant with past population expansion. This study will facilitate further investigations into the contribution of population-level processes to the formation and spread of adaptive traits and species formation in the group. Hopefully, the support for similar mechanisms of diversification in *Drosophila* and *Tetragnatha* will motivate studies of additional Hawaiian taxa and focus greater attention on population-level processes occurring across lava flows.

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Amy Vandergast (http://www.bio.sdsu.edu/eb/avanderg) is a postdoctoral researcher at the USGS San Diego Field Station and an adjunct assistant professor at San Diego State University. She investigates the influences of landscape and anthropogenic disturbance on the population genetic structure of terrestrial arthropods. Rosemary Gillespie (http://nature.berkeley.edu/~gillespi/) currently holds the Schlinger Chair of Systematics and is Professor and Director of the Essig Museum of Entomology at UC Berkeley. Her research focuses on diversification of spiders on remote oceanic islands of the Pacific. George Roderick (http://nature.berkeley.edu/~roderick) is Professor and Director of UC Berkeley Gump South Pacific Research Station (http://moorea.berkeley.edu). He is interested in the demographic history of populations and species, particularly terrestrial arthropods.

Appendix I

Tetragnatha quasimodo; allozyme allele frequencies for each locus and population sampled

		Population												
Locus		F1	F2	F3	F4	F5	F6	F7	F6	C1	C2	C3	C4	C5
PGI	N	19	15	15	33	20	13	12	13	6	16	19	19	4
	A	0	0	0.0333	0.0152	0	0	0	0	0.0833	0.125	0	0	0.125
	B	0.7895	0.7333	0.7667	0.7879	0.875	0.9231	0.8333	0.9231	0.75	0.7188	0.7632	0.8684	0.75
	C	0.2105	0.2667	0.2	0.197	0.125	0.0769	0.1667	0.0769	0.1667	0.1563	0.2368	0.1316	0.125
PGM	N	19	14	15	33	20	13	12	13	6	16	19	19	4
	A	0	0	0.0333	0	0.05	0	0	0	0	0.0313	0.0263	0.0263	0.125
	B	0.9737	1	0.9667	0.9697	0.85	1	0.9167	1	1	0.9375	0.9737	0.9211	0.0875
	C	0.0263	0	0	0.0303	0.1	0	0.0833	0	0	0.0313	0	0.0526	0
AATf	N	19	16	16	33	20	13	12	13	6	16	19	19	4
	A	0.0263	0	0	0	0	0	0	0	0	0	0	0	0
	B	0.9737	1	1	1	1	1	1	1	1	1	1	1	1
APKs	N	23	28	22	36	15	13	12	13	6	16	19	16	0
	A	1	0.9821	1	1	1	1	1	1	1	1	1	1	
	B	0	0.0179	0	0	0	0	0	0	0	0	0	0	
APKf	N	23	28	22	36	15	13	12	13	6	16	19	16	0
	A	1	1	1	1	1	1	1	1	1	1	1	1	
IDHs	N	15	25	20	35	20	13	11	13	6	16	17	14	4
	A	0.9667	0.98	0.925	1	0.975	0.8462	0.9545	0.8462	0.9167	0.9375	0.9706	0.8214	0.875
	B	0.0333	0.02	0.075	0	0.025	0.1538	0.0455	0.1538	0.0833	0.0625	0.0294	0. 1786	0.125
IDHf	N	23	28	22	36	20	13	12	13	6	16	19	19	4
	A	0	0	0.0455	0	0.025	0	0	0	0.1667	0.0313	0	0	0
	B	0.9783	0.9643	0.9318	1	0.975	1	0.9583	1	0.8333	0.9688	0.9737	1	1
	C	0.0217	0.0357	0.0227	0	0	0	0.0417	0	0	0	0.0263	0	0
MDH	N	23	28	22	36	20	13	12	13	6	16	19	18	4
	A	0	0	0	0	0.025	0	0	0	0	0	0	0.0278	0
	B	1	1	0.9545	1	0.975	1	1	1	0.8333	1	0.973 7	0.9722	1
	C	0	0	0.0455	0	0	0	0	0.1667	0	0.0263	0	0	
G3PDH	N	23	24	17	23	20	13	12	13	6	16	19	19	4
	A	0	0	0.0294	0	0	0	0	0	0	0	0	0	0
	B	1	1	0.9706	1	1	1	1	1	1	1	1	1	1

Appendix II

T. anuenue; allozyme allele frequencies for all loci and populations sampled

	Population										
Locus		F1	F2	F3	F4	F5	F6	F7	C2	C3	C4	C5
IDHs	N	23	12	27	53	20	11	16	17	18	21	31
	A	0.0217	0.0417	0	0.0189	0.05	0.0455	0.0313	0.0588	0.0556	0	0.0484
	B	0	0	0	0	0	0	0	0	0.0278	0	0
	C	0.9565	0.9167	0.9815	0.9717	0.95	0.9545	0.9688	0.9412	0.9167	1	0.9194
	D	0.0217	0.0417	0.0185	0.0094	0	0	0	0	0	0	0.0323
IDHf	N	23	26	43	65	20	13	17	17	18	18	31
	A	0	0	0	0	0	0	0	0.0294	0.0556	0	0
	B	1	0.9808	1	1	1	0.8846	0.9706	0.09706	0.9444	1	0.9839
	C	0	0.0192	0	0	0	0	0	0	0	0	0.0161
	D	0	0	0	0	0	0.1154	0.0294	0	0	0	0
6PGDH	N	23	12	30	45	19	13	16	17	18	19	31
	A	0.0652	0	0	0	0	0	0	0	0	0	0
	B	0.913	1	0.9833	0.9889	1	1	0.9688	1	1	1	0.9839
	C	0.0217	0	0.0167	0	0	0	0.0313	0	0	0	0.0161
	D	0	0	0	0.0111	0	0	0	0	0	0	0
MDH	N	22	21	43	71	20	13	17	17	11	22	29
	A	1	1	1	1	1	1	1	1	1	1	1
PGM	N	23	23	34	46	19	12	16	17	17	21	31
	A	0	0.0217	0.0588	0.0217	0	0.0417	0.0313	0.0588	0.0294	0.1190	0.0484
	B	0.09348	0.7391	0.8382	0.8804	0.9211	0.7500	0.9063	0.8824	0.9706	0.6667	0.871
	C	0.0652	0.2391	0.1029	0.0978	0.0789	0.1667	0.0625	0.0588	0	0.2143	0.0806
	D	0	0	0	0	0	0.0417	0	0	0	0	0
AAT	N	23	23	37	45	20	11	16	17	18	22	31
	A	0	0	0	0.0111	0.025	0	0	0	0	0	0
	B	0.6957	0.6087	0.5811	0.6778	0.675	0.55	0.625	0.5294	0.6944	0.5682	0.5968
	C	0	0	0	0	0.025	0	0	0	0	0	0
	D	0.3043	0.3913	0.4189	0.3111	0.275	0.45	0.375	0.4706	0.3056	0.4318	0.4032
APKs	N	23	20	43	69	20	14	17	17	18	21	31
	A	1	1	1	1	1	1	1	1	1	1	1
APKf	N	23	18	44	66	20	14	17	17	18	22	31
	A	1	1	1	1	1	1	1	1	1	1	1
G3PDH	N	23	14	37	69	20	14	17	17	11	21	31
	A	1	1	1	1	1	1	1	1	1	1	1
PGI	N	23	24	39	46	18	14	16	17	18	21	31
	A	0	0	0	0	0	0	0	0	0	0	0.0161
	B	0	0	0.0128	0	0	0	0	0	0	0	0
	C	0.2391	0.2708	0.2564	0.1304	0.1667	0.5	0.25	0.2353	0.1667	0.0714	0.2419
	D	0	0	0	0.0109	0	0	0	0	0	0	0
	E	0	0.0208	0	0.0217	0.0278	0	0.0313	0	0	0	0
	F	0.7391	0.6667	0.6667	0.8261	0.75	0.5	0.625	0.7059	0.8056	0.9286	0.7258
	G	0	0	0	0	0.0556	0	0	0	0	0	0
	H	0.0217	0.0417	0.0641	0.0109	0	0	0.0938	0.0588	0.0278	0	0.0161

Appendix III

T. brevignatha; allozyme allele frequencies for all loci and populations sampled

	Population								
Locus		F1	F2	F3	F5	C1	C2	C3	C4	C5
PGI	N	18	17	13	7	7	20	20	21	17
	A	0.0278	0.0294	0	0	0	0.025	0	0.0238	0
	B	0.9722	0.9412	0.9615	0.9286	1	0.975	0.975	0.9762	1
	C	0	0.0294	0.0385	0.0714	0	0	0.025	0	0
PGM	N	17	16	17	7	7	20	19	21	16
	A	0	0	0.0294	0	0.0714	0.025	0.0263	0	0
	B	0.8824	0.9688	0.8529	0.9286	0.9286	0.925	0.8684	1	0.9688
	C	0.1176	0.0313	0.1176	0.0714	0	0	0.1053	0	0.0313
IDHs	N	18	15	16	7	7	20	19	21	16
	A	0	0.0667	0.0313	0.0714	0	0.075	0.0789	0.0714	0.0313
	B	1	0.9333	0.9375	0.9286	0.9286	0.9	0.8684	0.9286	0.988
	C	0	0	0.0313	0	0	0.025	0.0263	0	0
	D	0	0	0	0	0	0	0.0263	0	0
	E	0	0	0	0	0.0714	0	0	0	0
IDHf	N	17	15	17	7	7	20	20	21	17
	A	1	1	1	1	1	0.975	1	0.9762	1
	B	0	0	0	0	0	0.025	0	0	0
	C	0	0	0	0	0	0	0	0.0238	0
MDH	N	18	13	13	7	7	20	20	20	17
	A	0	0	0	0.0714	0	0	0	0	0
	B	0.9722	0.9231	1	0.8571	1	1	1	1	0.9706
	C	0	0.0769	0	0.0714	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	0.0294
	E	0.0278	0	0	0	0	0	0	0	0
AAT	N	16	14	17	7	7	20	18	19	17
	A	1	1	1	1	1	0.975	1	1	1
	B	0	0	0	0	0	0.025	0	0	0
APKf	N	16	15	13	7	7	20	20	21	17
	A	1	1	1	1	1	1	1	1	1
APKs	N	18	17	13	7	7	20	20	21	17
	A	1	1	1	1	1	1	1	1	1
6PGDH	N	17	16	15	7	7	20	20	21	17
	A	0.2059	0.2813	0.2	0.3571	0.0714	0.15	0.1	0.119	0.0882
	B	0.6176	0.7188	0.7667	0.3571	0.9286	0.775	0.825	0.7857	0.8235
	C	0. 1765	0	0.0333	0.2857	0	0.05	0.075	0.0952	0.0882
	D	0	0	0	0	0	0.025	0	0	0
G3PDH	N	18	15	13	7	7	6	20	21	17
A	1	1	1	1	1	1	1	1	1	