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What is adaptive radiation? Many manifestations of the phenomenon in an iconic lineage of Hawaiian spiders

Susan R. Kennedy [a,*], Jun Ying Lim [b], Seira Ashley Adams [c], Henrik Krehenwinkel [a], Rosemary G. Gillespie [c]

[a] *Department of Biogeography, Universität Trier, Germany*
[b] *Department of Biological Sciences, National University of Singapore, Singapore*
[c] *Department of Environmental Science, Policy and Management, University of California Berkeley, USA*

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ABSTRACT

Adaptive radiation provides the ideal context for identifying and testing the processes that drive evolutionary diversification. However, different adaptive radiations show a variety of different patterns, making it difficult to come up with universal rules that characterize all such systems. Diversification may occur via several mechanisms including non-adaptive divergence, adaptation to novel environments, or character displacement driven by competition. Here, we characterize the ways these different drivers contribute to present-day diversity patterns, using the exemplary adaptive radiation of Hawaiian long-jawed orbweaver (*Tetragnatha*) spiders. We present the most taxonomically comprehensive phylogenetic hypothesis to date for this group, using 10 molecular markers and representatives from every known species across the archipelago. Among the lineages that make up this remarkable radiation, we find evidence for multiple diversification modalities. Several clades appear to have diversified in allopatry under a narrow range of ecological conditions, highlighting the role of niche conservatism in speciation. Others have shifted into new environments and evolved traits that appear to be adaptive in those environments. Still others show evidence for character displacement by close relatives, often resulting in convergent evolution of stereotyped ecomorphs. All of the above mechanisms seem to have played a role in giving rise to the exceptional diversity of morphological, ecological and behavioral traits represented among the many species of Hawaiian *Tetragnatha*. Taking all these processes into account, and testing how they operate in different systems, may allow us to identify universal principles underlying adaptive radiation.

1. Introduction

Adaptive radiation, the evolutionary divergence of members of a single lineage into a variety of adaptive forms (Futuyma, 1998), has been considered the lynch-pin that unites ecology and evolution (Givnish & Sytsma, 1997). The nature of adaptive radiations can vary greatly among systems, making it difficult to draw generalities. One of the few common denominators is that adaptive radiations tend to require some form of ecological opportunity which can then allow for divergent ecological selection (Schluter, 2000a; Stroud & Losos, 2016). However, the mechanisms that drive subsequent diversification have been extensively debated and many factors have been implicated (Baguette et al., 2020; Dorey et al., 2020; Ronco et al., 2021). As a result, there is still limited understanding of universal principles underlying the processes that shape these systems.

Several mechanisms of diversification have been suggested as playing a role in adaptive radiation, and these mechanisms vary depending on the role of the environment versus interactions between close relatives (Gillespie et al., 2020). Under **non-adaptive diversification** (***Scenario 1***)**,** the geography of the environment has been implicated as an initial factor in population divergence and species formation (Goodman et al., 2019; Heinicke et al., 2017; Schenk & Steppan, 2018). In this case, species become isolated by geographic barriers, but do not undergo divergent selection; instead, they retain their ancestral niche (“niche conservatism”; Wang et al. 2021). The result is a classic “non-adaptive” radiation (e.g., Rundell & Price, 2009) with little ecological disparity between members of the radiation. Although this scenario does not fit under the strict definition of adaptive radiation, we include it here because it has been shown to play an important role in species diversification in many groups, e.g. *Albinaria* land snails (Gittenberger, 1991),

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* Corresponding author at: Universität Trier, Fachbereich VI - Raum- und Umweltwissenschaften, Fach Biogeographie, 54296 Trier, Germany.
E-mail addresses: kennedy@uni-trier.de, susanrkennedy@gmail.com (S.R. Kennedy).

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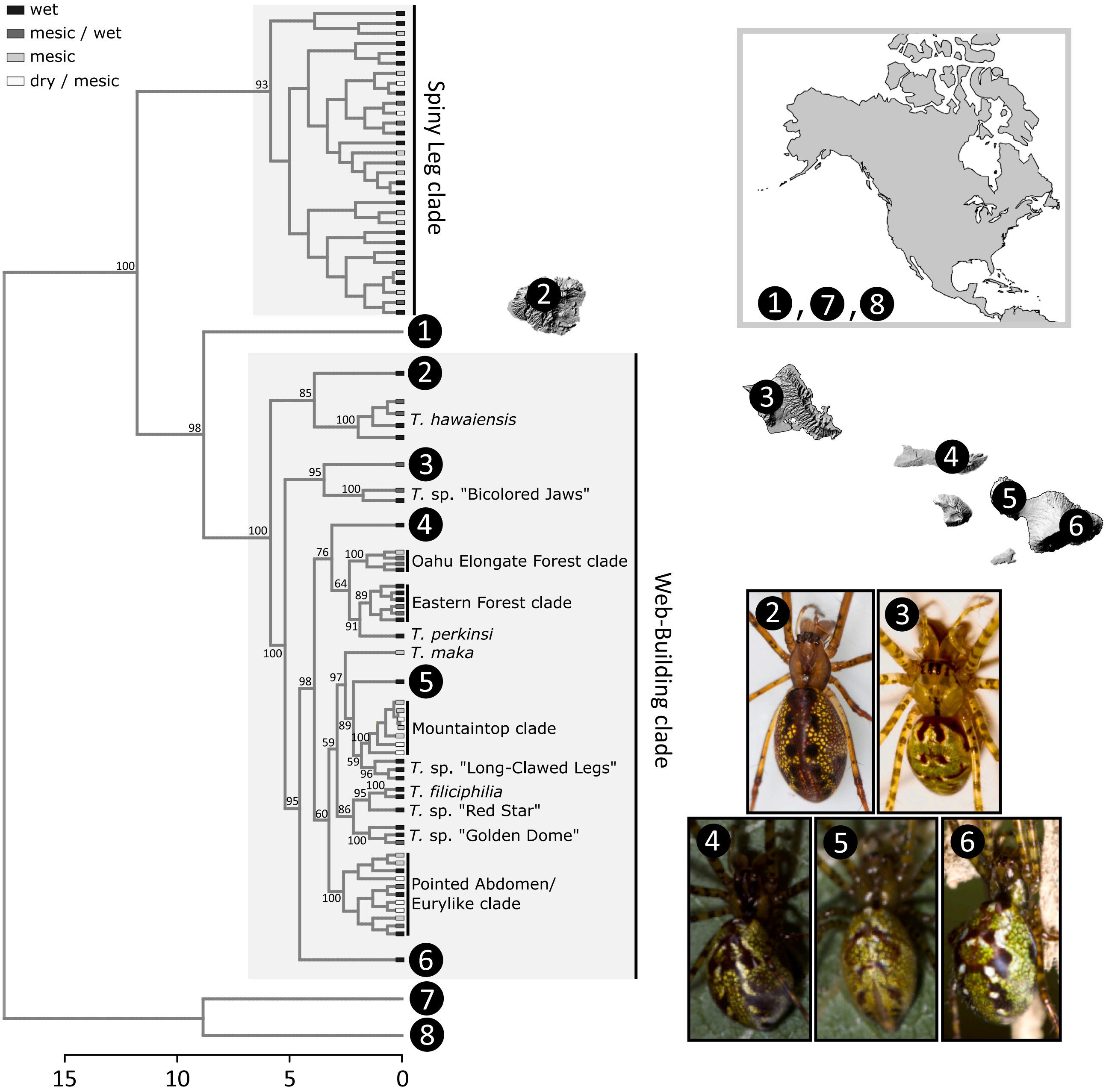

Fig. 1. Evolutionary relationships among clades. Maximum likelihood phylogeny of Hawaiian *Tetragnatha* species, showing major clades. Spiders with the “paludicola-like” ecomorphology, restricted to wet forests and distinguished by a large, rounded abdomen and bright green coloration, are indicated by numbers 2–6. Numbers 1, 7 and 8 are outgroup species distributed in North America. 1 = *T. pallescens*, 2 = *T.* sp. “Waves” from Kauai, 3 = *T.* sp. “Emerald Ovoid” from Oahu, 4 = *T. paludicola* from Molokai, 5 = *T. paludicola* from West Maui, 6 = *T. paludicola* from East Maui, 7 = *T. versicolor*, and 8 = *T. viridis*. Bootstrap support values for the major clades and species outside of these clades are shown to the left of nodes. Scale bar shows relative dates in millions of years based on *chronos*, using the geologic age of Kauai (Clague & Sherrod, 2014) to calibrate the ages of nodes basal to both the Spiny Leg and web-building clades (see section 2.4). Tip shades indicate moisture level of the habitat occupied by each taxon, based on the Carbon Assessment of Hawaii/Land Cover/Biome Unit dataset of the Hawaii Statewide GIS Program (Jacobi et al. 2017), from wet (dark gray) to dry/mesic (light gray). Relief map of Hawaii obtained from EROS Data Center, National Atlas of the United States. Spider photos by George Roderick and Rosemary Gillespie. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

North American two-lined salamanders (Kozak et al., 2006) and *Orsonwelles* sheet web spiders (Hormiga et al., 2003).

In other situations, the formation of new species may be shaped by allopatric or parapatric **adaptation to novel environments** (***Scenario 2***). For example, some fish lineages show evidence of divergence associated with moving from a littoral/benthic to a pelagic/limnetic lifestyle, or vice versa: the juxtaposition of these disparate habitat types appears to lead to disruptive or divergent selection, which initiates the radiation (Hendry et al., 2009; Maan et al. 2017; Martin & Wainwright, 2013). Likewise, elevation zones pose physiological barriers to many organisms, and radiations may occur when divergence is associated with adaptation to different elevational extremes (De Busschere et al., 2010). A similar phenomenon has been reported in cave fauna, with lineages undergoing repeated adaptation to, and diversification within, the subterranean environment (Hoch, 1997). These types of processes are often associated with repeated evolution of ecotypes and species that diverge in response to similar sets of environmental contrasts (Walter et al., 2018).

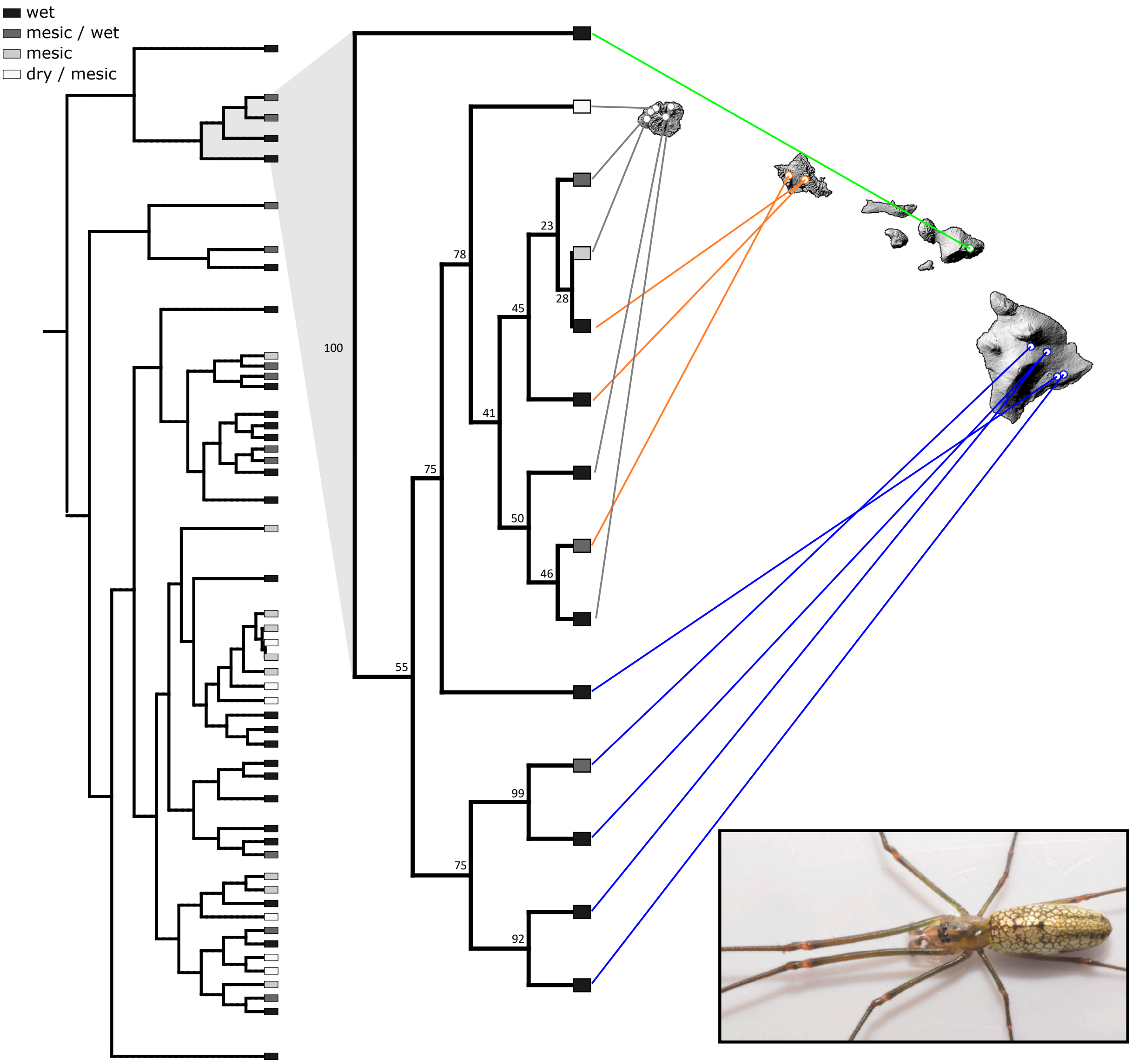

Fig. 2. Scenario 0 – No diversification. Maximum Likelihood phylogeny of *T. hawaiensis*, showing its position within the clade of Hawaiian web-builders. Lines from each tip indicate the locality from which that specimen was collected; the color of each line corresponds to an island or island complex (gray = Kauai, orange = Oahu, green = Maui Nui, blue = Big Island). Inset: Closeup photo of *T. hawaiensis*. Bootstrap support values are shown to the left of each node. Tip shades indicate moisture level of the habitat occupied by each population, based on the Carbon Assessment of Hawaii/Land Cover/Biome Unit dataset of the Hawaii Statewide GIS Program (Jacobi et al. 2017), from wet (dark gray) to dry/mesic (light gray). Relief map of Hawaii obtained from EROS Data Center, National Atlas of the United States. Spider photo by Susan Kennedy. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

A third potential driver of adaptive radiation is **character displacement** (***Scenario 3***) (Schluter, 2000b). This begins in a similar manner to Scenario 1 in that populations first become isolated in locations that have a similar environment, and evolve intrinsic reproductive barriers and/or genetic incompatibilities during the period of isolation. However, in this case, the divergent entities later return to sympatry, for example if their ranges become connected by suitable habitat. At first, the species will be ecologically virtually identical, and interactions between close relatives will therefore lead to strong competition. This competition pressure will select for character displacement, leading to niche divergence between co-occurring species (Brown & Wilson, 1956). This type of scenario appears to be associated with repeated evolution of ecomorphs which co-occur only with ecologically divergent relatives, presumably because the divergent selection is for niche shifts between close relatives within a given environment (Gillespie et al., 2018; Zakšek et al., 2019). While all of these scenarios can contribute to diversification, much is still unknown about the relative importance of each scenario in giving rise to species within a single adaptive radiation.

The current paper aims to test these scenarios by inferring the mechanisms underlying species formation in the context of a single adaptive radiation. Specifically, if a radiation is characterized as being (1) “non-adaptive,” then we would expect diversification to occur entirely in allopatry or parapatry and within the same environmental regime; (2) associated with adaptation to novel environmental conditions, then we expect close relatives to be in different environments that are largely allo- or parapatric; moreover, if this is a common mode of diversification, we expect repeated evolution of ecotypes associated with similar sets of environments in different localities; and (3) associated with niche shifts due to interactions between close relatives, then we expect sister species to co-occur at a site, but to occupy different niches; furthermore, in this situation we expect repeated evolution of ecomorphs associated with similar sets of interactions and niches that arise independently in different locations. We test these three scenarios in a speciose adaptive radiation of spiders in Hawaii.

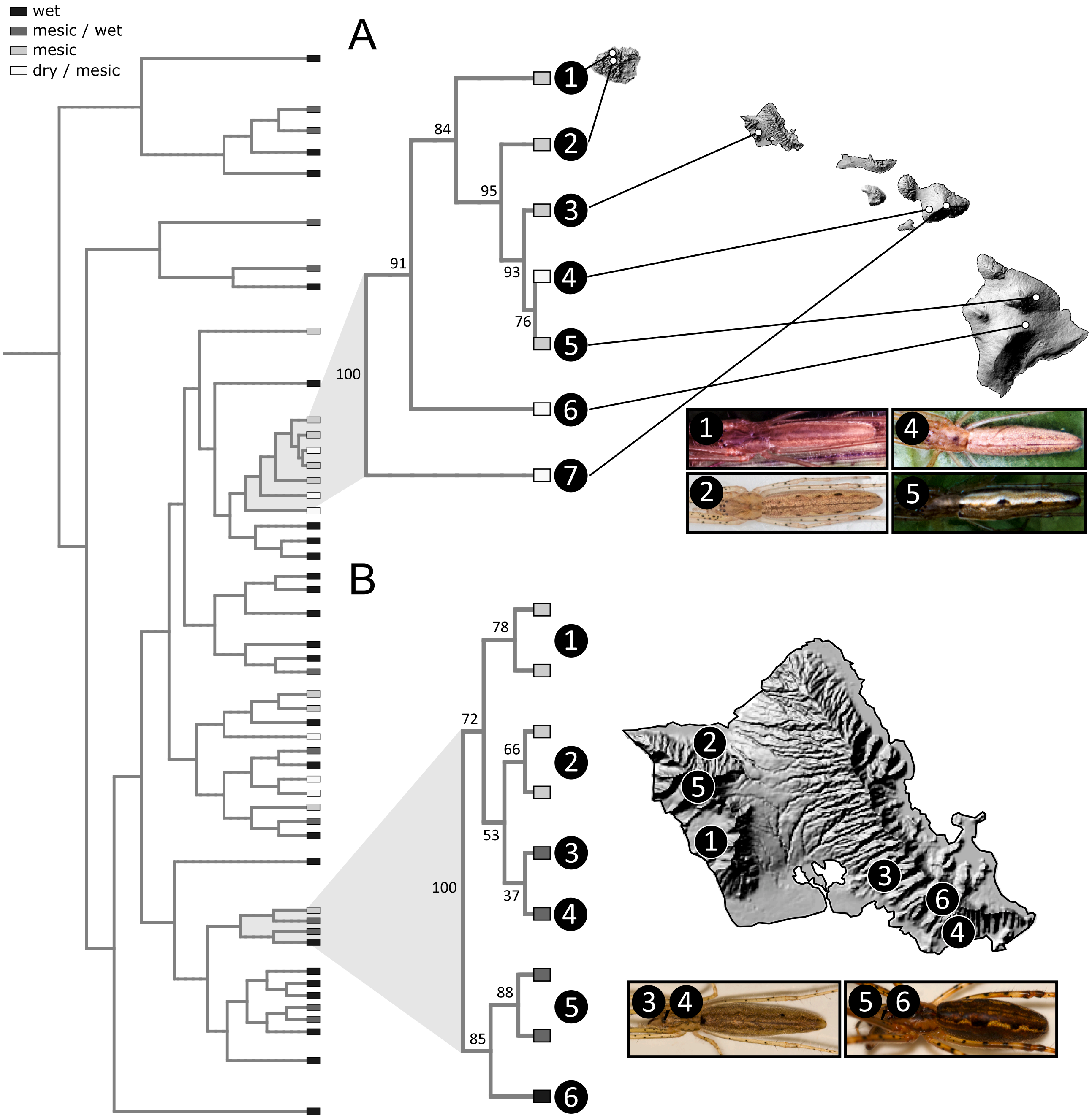

Fig. 3. Scenario 1 – Nonadaptive diversification in web-builders. Clades of web-building Hawaiian *Tetragnatha* that exhibit nonadaptive diversification, showing their positions within the web-building clade (Maximum Likelihood phylogeny). Numbers on islands indicate localities where specimens were collected. A) The Mountaintop clade; 1 = undescribed species from Waiahuakua (Kauai), 2 = undesc. sp. from Mohihi-Waialae (Kauai), 3 = *T. uluhe* from Halona (Oahu), 4 = undesc. sp. from Auwahi (Maui), 5 = undesc. sp. from Hakalau (Big Island), 6 = undesc. sp. from Pu'u Huluhulu (Big Island), 7 = undesc. sp. from Haleakalā Crater (Maui). B) The Oahu Elongate Forest clade; 1 = *T. palikea* from low Palikea, 2 = *T. palikea* from low Mt. Ka'ala, 3 = *T. lena* from low Poamoho, 4 = *T. lena* from low Mt. Tantalus, 5 = *T. limu* from the summit of Mt. Ka'ala, 6 = *T. limu* from the summit of the Koolaus. Bootstrap support values are shown to the left of each node. Tip shades indicate moisture level of the habitat occupied by each population, based on the Carbon Assessment of Hawaii/Land Cover/Biome Unit dataset of the Hawaii Statewide GIS Program (Jacobi et al. 2017), from wet (dark gray) to dry/mesic (light gray). Relief map of Hawaii obtained from EROS Data Center, National Atlas of the United States. Spider photos by George Roderick and Rosemary Gillespie.

The Hawaiian Islands make up a remarkable geological chronosequence, arranged linearly from oldest to youngest: Kauai (~5 my old) is the oldest and northwesternmost island, followed by Oahu (4.3 my old), the island complex of Maui Nui (Maui, Molokai, Lanai and Kahoolawe; 2.1 my old), and finally the Big Island of Hawaii (~1 my old; Clague & Sherrod, 2014). This chronological arrangement makes it possible to track evolutionary patterns across slices in geological time (Lim & Marshall, 2017; Shaw & Gillespie, 2016). Owing to its extreme isolation (Emerson & Gillespie, 2008), as well as the separation of the islands from one another and the many within-island barriers created by volcanic activity (Bennett & O'Grady, 2013), Hawaii has given rise to numerous adaptive radiations. Famous examples include the honeycreepers (Lerner et al., 2011), multiple groups of insects including flies (Magnacca & Price, 2015), moths (Haines et al., 2014) and crickets (Mendelson & Shaw, 2005), and plants such as silverswords (Baldwin et al., 2021; Landis et al., 2018) and lobeliads (Givnish et al., 2009). We

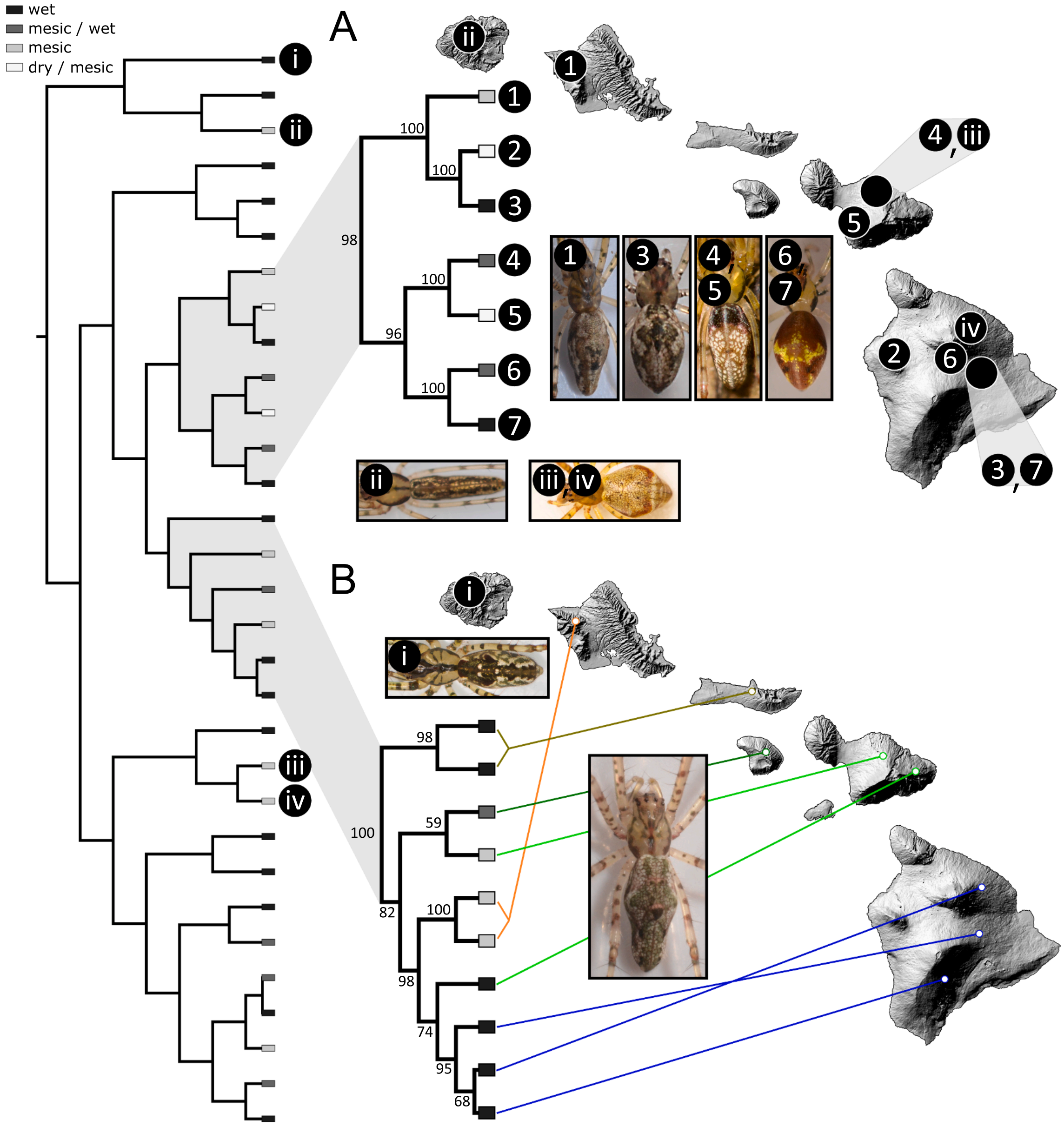

Fig. 4. Scenario 1 – Nonadaptive diversification in Spiny Leg spiders. Clades of Spiny Leg *Tetragnatha* that exhibit nonadaptive diversification, showing their positions within the Spiny Leg clade (Maximum Likelihood phylogeny). A) The Small Spiny clade. Numbers on islands indicate localities where specimens were collected: 1 = *T. kukuiki* from Pahole (Oahu), 2 = *T. kukuhaa* (not pictured) from Pu'u Waawaa (Big Island), 3 = *T. obscura* from Pu'u Maka'ala (Big Island), 4 = *T. kikokiko* from Lower Waikamoi (Maui), 5 = *T. kikokiko* from Auwahi (Maui), 6 = *T. anuenue* from the kipukas on Saddle Road (Big Island), 7 = *T. anuenue* from Pu'u Maka'ala. Two other species belonging to the Small Brown ecomorph fall outside of this clade: *T. mohihi* (ii) on Kauai and *T. restricta* (iii and iv) on Maui and the Big Island, respectively. B) *T. quasimodo* (center photo), of the Large Brown ecomorph. Lines from each tip indicate the locality from which that specimen was collected; color of each line corresponds to an island (orange = Oahu, tan = Molokai, dark green = Lanai, light green = Maui, blue = Big Island). Lineages are largely structured by island. *T. quasimodo* is present on all islands except Kauai, where *T. pilosa* (i), also of the Large Brown ecomorph, occurs. Bootstrap support values are shown to the left of each node. Tip shades indicate moisture level of the habitat occupied by each taxon, based on the Carbon Assessment of Hawaii/Land Cover/Biome Unit dataset of the Hawaii Statewide GIS Program (Jacobi et al. 2017), from wet (dark gray) to dry/mesic (light gray). Relief map of Hawaii obtained from EROS Data Center, National Atlas of the United States. Spider photos by George Roderick, Rosemary Gillespie and Susan Kennedy. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

focus on the long-jawed orbweaver spiders (Tetragnathidae: *Tetragnatha* spp.), a diverse adaptive radiation with an estimated 60 species (Gillespie, 2016) belonging to two major clades: the web-builders, which capture their prey using a two-dimensional orb web, and the Spiny Leg clade, which hunt actively. The vast majority of species within the radiation are single-island endemics. This exceptional group of spiders encompasses a wide range of morphological (Gillespie 2016), ecological (Gillespie 2004) and behavioral traits (Kennedy et al. 2019), which stand in contrast to the relatively conserved morphology and natural history of congeners throughout the rest of *Tetragnatha*'s global distribution (Alvarez-Padilla & Hormiga, 2011). Alongside this striking diversity, the Hawaiian *Tetragnatha* also show evidence for convergent evolution of morphologies (ecomorphs) associated with specific substrate types in the Spiny Leg clade (Gillespie, 2004), and of certain web structures in the web-building clade (Blackledge & Gillespie, 2004).

In order to understand the common denominators underlying diversification in this lineage, it is critical to have a well-supported phylogenetic hypothesis for the entire Hawaiian radiation. While some

Table 1

List of DNA barcode markers sequenced to generate phylogenetic data. Amplicon lengths presented in the second column include the primer-binding sites.

Locus	Amplicon length (bp)	Compartment	Protein-coding?	F Primer	Sequence 5′ to 3′	Source
Cytochrome Oxidase I (COI)	467	mitochondrial	yes	ArF1	GCNCCWGAYATRGCNTTYCCNCG	(Gibson et al., 2014)
				Fol-degen-rev	TANACYTCNGGRTGNCCRAARAAYCA	(Yu et al., 2012)
16SrDNA (16S)	401	mitochondrial	no	16SF3	CGRTYTRAACTCAGATCATGTA	(Krehenwinkel et al., 2018)
				16SR1	TRACYGTRCWAAGGTAGCATAA	(Krehenwinkel et al., 2018)
12SrDNA (12S)	424	mitochondrial	no	12SF1	NCHACTWTGTTACGACTT	(Krehenwinkel et al., 2018)
				12SR1	AMTAGGATTAGATACCCT	(Krehenwinkel et al., 2018)
Cytochrome B (CytB)	406	mitochondrial	yes	CB3degB	GAGGDGCHACHGTWATYACHAA	(Barraclough et al., 1999; Krehenwinkel et al., 2018)
				CB4deg	RAARTATCATTCDGGTTGRATRTG	(Barraclough et al., 1999; Krehenwinkel et al., 2018)
18SrDNA (SSU)	421	nuclear	no	SSU_F04	GCTTGTCTCAAAGATTAAGCC	(Fonseca et al., 2010)
				SSU_R22	GCCTGCTGCCTTCCTTGGA	(Fonseca et al., 2010)
18SrDNA (18SM)	351	nuclear	no	18s_2F	AACTTAAAGRAATTGACGGA	(Machida & Knowlton, 2012)
				18s_4R	CKRAGGGCATYACWGACCTGTTAT	(Machida & Knowlton, 2012)
28SrDNA (28SM)	363	nuclear	no	28s_3F	TTTTGGTAAGCAGAACTGGYG	(Machida & Knowlton, 2012)
				28s_4R	ABTYGCTACTRCCACYRAGATC	(Machida & Knowlton, 2012)
Histone H3 (H3)	374	nuclear	yes	H3aF	ATGGCTCGTACCAAGCAGACVGC	(Colgan et al., 1998)
				H3aR	ATATCCTTRGGCATRATRGTGAC	(Colgan et al., 1998)
ITS2	436	nuclear	no	5.8S3F	ATCACTHGGCTCRYGGRTCGATG	(Krehenwinkel et al., 2018)
				28S2R	TTCTTTTCCTCCSCTHANTDATATGC	(Krehenwinkel et al., 2018)
Actin	271	nuclear	yes	Actin_F2	GAYTTYGARCARGARATGGCNAC	This paper
				Actin_R1	GRTCDGCAATNCCWGGRTACAT	This paper

clades within the Hawaiian *Tetragnatha* have been well documented in terms of overall diversity, ecological affinity, behavior and morphology (Binford et al., 2016; Blackledge & Gillespie, 2004; Gillespie, 2004; Kennedy et al., 2019), to date there has not been a comprehensive, multi-locus phylogenetic analysis including every known species. In particular, although evolutionary relationships within the Spiny Leg clade are well characterized (Gillespie, 2004), the phylogeny of the web-building clade remains poorly understood. Here, we present the most taxonomically complete and data-rich phylogenetic analysis of the Hawaiian *Tetragnatha* to date, based on six nuclear and four mitochondrial markers (Krehenwinkel et al., 2018), and test the scenarios for diversification outlined above. Our results highlight the variety of processes that have led to the rise of the many species within this noteworthy group of spiders.

2. Materials and methods

2.1. Specimen collection and storage

Spiders were individually hand-collected and preserved in 95 % ethanol. For each Hawaiian *Tetragnatha* species, specimens were sampled from across the species' known range, and we strove to include a minimum of one specimen per species per site (Supplementary Table 1). Outgroup specimens were collected from the continental USA. Specimens were collected under permits from the State of Hawaii Department of Land and Natural Resources (endorsement #s FHM13-313, FHM14-339, FHM14-349 and FHM17-422) and the National Park Service (study # HAVO-00425). After DNA extraction (see section 2.2), specimens were vouchered at the UC Berkeley Essig Museum of Entomology.

To ensure comprehensive coverage of all species or populations, we augmented our sampling with sequence data from previous studies (Gillespie, 1999, 2005; Gillespie et al., 1997; Gillespie et al., 1994). Our final dataset consisted of sequence data from 107 individuals representing 55 putative species. Of these, 84 individuals were used in the main phylogenetic analysis, while the remaining 23 were used to increase the population-level resolution of sub-analyses of smaller clades (*T. hawaiensis*, Fig. 2; the Oahu Elongate Forest clade, Fig. 3B; *T. quasimodo*, Fig. 4B).

2.2. DNA extraction, PCR and sequencing

DNA was extracted from leg tissue of individual specimens, using the Qiagen PureGene tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Ten DNA markers (Table 1) were amplified with multiplex PCR, using a 10-μL volume and the Qiagen Multiplex PCR kit (see Supplementary Table 2 for protocols). All primers included Illumina TruSeq tails on the 5′ end, enabling a second round of PCR in which the sequencing primers and a unique combination of forward and reverse indexing barcodes were added to the amplicons for each sample as in Lange et al. (2014). Indexed PCR products were visualized on a 1.5 % agarose gel and pooled in approximately equimolar amounts based on gel band intensity. The pooled product was cleaned of residual primers with 1X AMPure beads XP (Beckman Coulter, Indianapolis, IN, USA) and quantified with a Qubit dsDNA high-sensitivity assay (Invitrogen, Carlsbad, CA, USA). The cleaned pool was sequenced at the California Academy of Sciences' Center for Comparative Genomics (San Francisco, CA, USA) on an Illumina MiSeq (Illumina) using V3 chemistry with 600 cycles.

2.3. Sequence processing

Sequences were demultiplexed by index barcode combination on the BaseSpace server (Illumina). Forward and reverse reads were merged using Paired-End reAd mergeR (PEAR) version 0.9.11 (Zhang et al., 2014) with a minimum quality of 20 and a minimum overlap of 50 bp. Merged reads were then quality filtered so that only those with at least Q30 quality on at least 90% of their length were retained, and these were converted to Fasta format using FastX-Toolkit version 0.0.13 (Gordon & Hannon, 2010). We used grep and sed in Unix to search for the F and R primer sequences in these Fasta files in order to demultiplex them by marker and trim off primer sequences. Demultiplexed and primer-trimmed sequences were then dereplicated and clustered into 3 % radius OTUs (97 % sequence similarity, corresponding roughly to the species level; Edgar, 2013), using USEARCH version 10.0.240 (Edgar, 2010). A *de novo* chimera removal step was included in the OTU clustering. OTUs were taxonomically identified by BLAST searching (Altschul et al., 1990) against the NCBI nucleotide database (accessed 04/2018), and those that did not match *Tetragnatha* sp. were discarded. NUMTs – fragments of mitochondrial DNA that have been transposed into the nuclear genome (Lopez et al., 1994) and are unintentionally coamplified by PCR targeting mitochondrial markers – were filtered out of the protein-coding mitochondrial genes (*COI* and *Cytochrome B*) by checking the translated sequences in MEGA7 (Kumar et al., 2016) and removing those with stop codons. If after these filtering steps, a specimen still had more than one OTU, the correct OTU was identified by running a neighbor-joining tree in MEGA7 and discarding OTUs that fell outside of clades formed by conspecifics.

2.4. Phylogenetic analyses

Sequences for each marker were aligned separately in MEGA7, using ClustalW (Thompson et al., 1994) with a transition weight of 0.5, a gap opening penalty of 15 and a gap extension penalty of 6.66. Alignments were uploaded to GenBank (accession numbers ON064384 – ON064985). The markers *28SrDNA*, *ITS2*, *12SrDNA* and *16SrDNA* contained hypervariable sites, so these four markers were concatenated by hand in MEGA7 and then run through the GBlocks server version 0.91b (Castresana, 2000) with “Maximum Number Of Contiguous Non-conserved Positions” set to 4, “Minimum Length Of A Block” set to 10, and “Allowed Gap Positions” set to “With Half.” The output was then concatenated with the remaining alignments by hand in MEGA7, resulting in a final alignment of 3273 bp. This was converted to phylip format in Geneious version 5.1.7 (https://www.geneious.com).

The best partitioning scheme (Supplementary Table 3) was identified using the Windows desktop version of PartitionFinder 2.1.1 (Lanfear et al., 2017), using “branchlengths = linked,” “models = all,” “model_selection = aicc,” and “search = greedy” (Lanfear et al., 2012). The analysis included the use of PhyML version 3.0 (Guindon et al., 2010). The resulting scheme was converted to nexus format in Notepad++ version 7.9.1.0 and then inputted into the desktop version of IQ-TREE 1.6.12 (Chernomor et al., 2016; Nguyen et al., 2015) with the alignment, using Ultrafast bootstrapping (Minh et al., 2013), to produce a Maximum Likelihood phylogeny. Bayesian analysis was performed in MrBayes 3.2.6 (Ronquist et al., 2012) on the CIPRES Science Gateway version 3.3 (Miller et al., 2010) with 10 million generations, using the same best partitioning scheme (Supplementary Table 3). Trees were visualized in FigTree 1.4.4 (https://tree.bio.ed.ac.uk/software/figtree/) and formatted in R 4.0.3 (R Core Team 2020) with RStudio 1.3.1093 (RStudio Team 2020), using *ape* version 5.4–1 (Paradis & Schliep, 2019) and *treeio* version 1.15.2 (Wang et al., 2020). Trees were made ultrametric using the *chronos* function in *ape* by applying minimum and maximum age constraints on the most recent common ancestors of both the Spiny Leg and web-building clades. We set the age range of these nodes to between 5.8 and 5.9 my: the age of the oldest exposed lava on Kauai (Clague & Sherrod, 2014) and the time of inception of Kauai island formation on the sea floor, respectively, following Lim & Marshall (2017). Cross-validation was performed by fitting ten smoothing values (lambda) between 0 and 1, separated by values of 0.1, and calculating their log-likelihood. Based on this calculation, we selected lambda = 0, which had the highest log-likelihood.

Smaller datasets for *T. hawaiensis* (Fig. 2), the Oahu Elongate Forest clade (Fig. 3B) and *T. quasimodo* (Fig. 4B) were analyzed in the same manner as above. Best partitioning schemes for each of these datasets are summarized in Supplementary Table 3.

3. Results and discussion

3.1. New insights into evolutionary relationships among the Hawaiian Tetragnatha

With the addition of many new taxa and new molecular markers, our results lend support to previous phylogenetic hypotheses for specific clades within the Hawaiian *Tetragnatha* radiation (Blackledge & Gillespie, 2004; Casquet et al., 2015; Gillespie, 2004; Gillespie et al., 1994), while also highlighting the evolutionary relationships within and among each of the clades within this exceptionally diverse group. Our results add support to an earlier finding (Gillespie et al., 1994) that the Spiny Leg and web-building clades likely arose from separate colonization events: the web-builders are more closely related to the continental species *T. pallescens* than to the Spiny Leg clade (Fig. 1). Species designations, which have thus far been based entirely on morphology and ecology, are largely held up. The widespread generalist species *T. hawaiensis*, which is found on all Hawaiian islands, diverges early in the web-building clade, supporting previous findings (Blackledge & Gillespie, 2004; Gillespie et al., 1997).

The current analysis reveals intriguing new patterns that differ across lineages. Early-diverging lineages of web-builders are characterized by a set of paraphyletic species that appear remarkably similar, with a large, rounded abdomen, often with bright iridescent green coloration (Fig. 1). Moreover, most are rare and narrowly distributed in wet, high elevation habitats. *T.* sp. “Emerald Ovoid” is limited to the mountain summits of Oahu, on both the Koolau and Waianae ranges. Likewise, the paraphyletic *T. paludicola* is restricted to high elevation wet forests of Molokai and Maui. Only the Kauai species *T.* sp. “Waves” has been found at relatively lower elevations (400 – 1200 m above sea level), also in wet forest. Interestingly, each of these taxa is subtended by a long branch, suggesting that they may be relicts.

Within each of the two major radiations, our analysis suggests that different drivers of diversification have acted on different lineages, leading to a complex and varied set of phenomena accounting for the extraordinary diversity of Hawaiian *Tetragnatha* spiders we see today.

3.2. Scenario 0: No diversification

Before addressing the potential drivers of diversification, we note one exception to the patterns that generally characterize the Hawaiian *Tetragnatha* radiation: namely, *T. hawaiensis*. Unlike most Hawaiian *Tetragnatha* species, which are endemic to a single island or island complex, *T. hawaiensis* is present on all the Hawaiian Islands (Fig. 2). This species is found primarily in wet forest, but mostly at low elevations (Gillespie et al., 1994), except on the youngest island of Hawaii, where it has been found as high as 1800 m. For this species, the overall higher connectivity of low elevation habitats (Price, 2004; Verboom et al., 2015) may have facilitated a greater level of exchange between islands.

The remaining lineages within the Hawaiian *Tetragnatha* radiation display marked diversification along geographic, morphological and ecological axes, with different apparent drivers of diversification in each case.

3.3. Scenario 1: Nonadaptive diversification

We first consider the mode of diversification wherein taxa diverge between different geographic areas without evident morphological or ecological change. The result is a lineage of genetically divergent taxa that are geographically separated, yet are morphologically similar and tend to occupy the same habitat type and/or niche. This pattern is evident in multiple groups where sister taxa show “niche conservatism” (Pyron et al., 2015; Wiens et al., 2010). Among the web-building *Tetragnatha*, the Mountaintop clade (Fig. 3A) is made up of species that appear to have originated on Kauai with a taxon found in mesic forest at elevations of 400 – 1300 m; on all other islands, these species are restricted to relatively dry habitats (mean annual rainfall 750 – 1200 mm; Giambelluca et al., 2013), whether in dry forest (leeward sides of Oahu, Maui and Hawaii) or high elevation scrubland above the inversion layer. Similar distributions are known in other arthropod lineages, such as Hawaiian *Thyrocopa* moths, where close relatives are limited to sites of low precipitation (Medeiros et al., 2015). All the species in the Mountaintop clade share a similar morphology characterized by a cigar-shaped body, long legs and brownish coloration. The clade is relatively young, with short branch lengths suggesting little genetic differentiation among species.

A somewhat similar pattern is apparent in the “Oahu Elongate

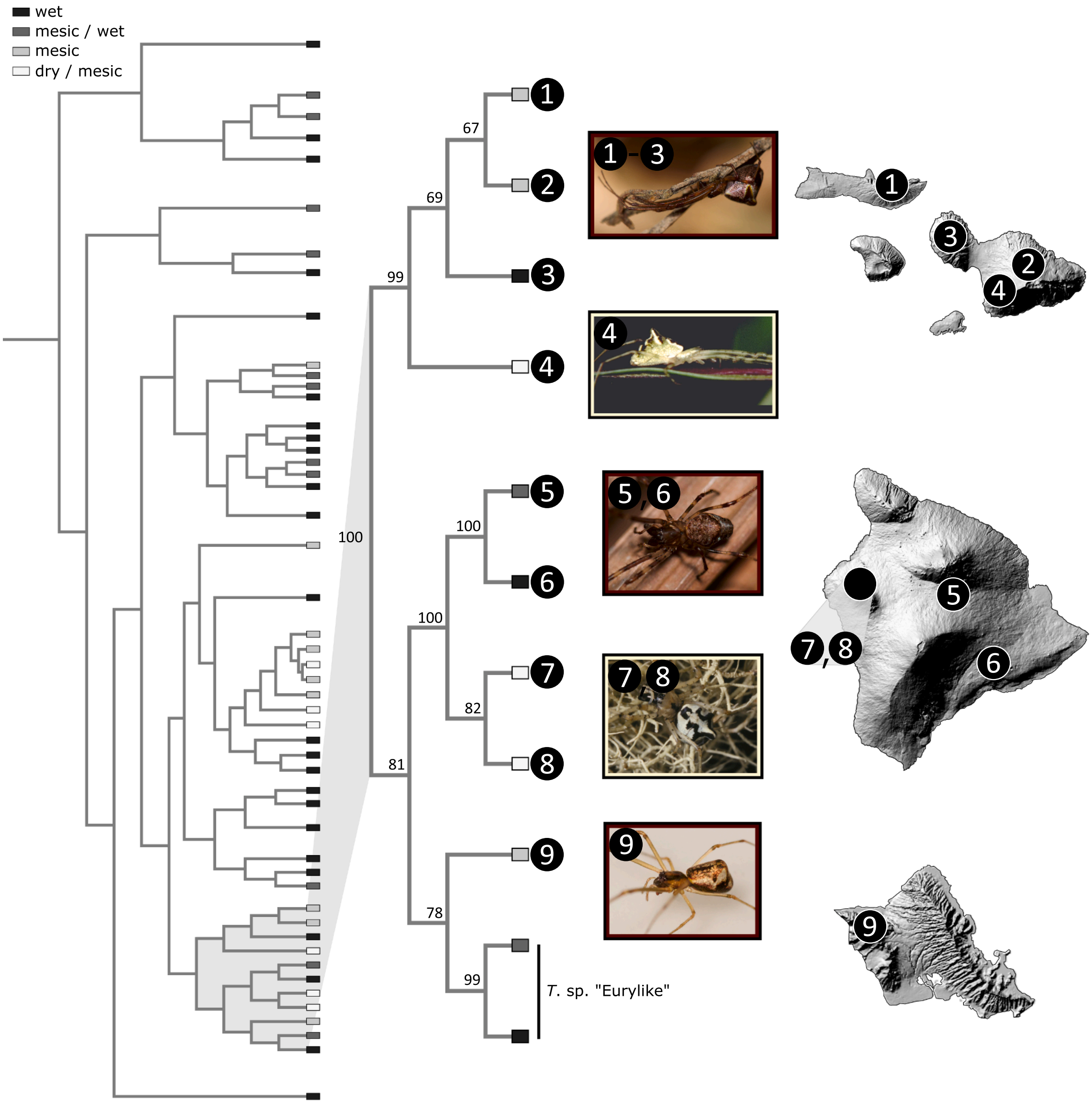

Fig. 5. Scenario 2 – Adaptation to novel environments. The Pointed Abdomen / *T.* sp. “Eurylike” clade, which shows evidence for adaptation to novel environments, showing its position within the web-building clade (Maximum Likelihood phylogeny). Numbers on islands indicate the localities where specimens were collected: 1–3 = *T. acuta* from Maui Nui (collected from the following sites: 1 = Kamakou (Molokai), 2 = Waikamoi (Maui), 3 = Pu’u Kukui (Maui)); 4 = *T. albida* from Auwahi (Maui); 5–6 = *T. acuta* from the Big Island (collected from 5 = kipukas along Saddle Road, 6 = Volcanoes National Park); 7–8 = the Big Island endemic *T. kea* (collected from 7 = Hualalai and 8 = Pu’u Waawaa); 9 = *T.* sp. “Pointed Abdomen” from Ohikilolo Ridge (Oahu). Bootstrap support values are shown to the left of each node. Tip shades indicate moisture level of the habitat occupied by each taxon, based on the Carbon Assessment of Hawaii/Land Cover/Biome Unit dataset of the Hawaii Statewide GIS Program (Jacobi et al. 2017), from wet (dark gray) to dry/mesic (light gray). Relief map of Hawaii obtained from EROS Data Center, National Atlas of the United States. Spider photos by George Roderick and Rosemary Gillespie.

Forest” clade consisting of *T. lena*, *T. limu* and *T. palikea* (Fig. 3B). These three species are all endemic to Oahu and, while the lineage has diverged into low-elevation and high-elevation clades, initial divergence between species (or populations) seems to have occurred geographically within the same habitat type. Thus, *T. lena* is geographically structured across low-elevation mesic (ca. 1500 – 2000 mm mean annual rainfall) forest on the Koolau volcano, and is sister to *T. palikea* which is found at similarly low elevations, but on the Waianae volcano (Gillespie, 2003). Similarly, *T. limu* is highly structured geographically, though in this species populations are limited to summits, with those on the summit of the Koolau mountains being sister to populations limited to the summits of the Waianae mountains; these summit habitats are characterized as “wet cliff,” with low scrub vegetation on poorly drained soils (Wagner et al., 1990). It therefore appears that closest relatives (*T. lena* and *T. palikea*) share similar habitat affinities. Morphology and niche have remained highly conserved in this clade, with all three species sharing an elongate body shape and building orb webs close to the ground (Gillespie, 2003).

The nature of niche conservatism varies between lineages. While the aforementioned clades within the web-builders exhibit this phenomenon at the site scale, lineages within the Spiny Leg clade tend to show niche conservatism at the microhabitat scale. Among the cursorial “spiny leg” lineage, one entire section, the “small spiny” clade (*T. anuenue*, *T. kikokiko*, *T. kukuhaa*, *T. kukuiki* and *T. obscura*), has speciated extensively between islands (Fig. 4A), yet the taxa have maintained their niche, living on twigs. Most of these species are confined to mesic or dry forest, though some are also found in wet forest on the Big Island (Gillespie et al., 2008). Thus, the species inhabit similar fine-scale niches and have similar morphologies, but the clade shows broad environmental tolerances on the youngest island. In the same

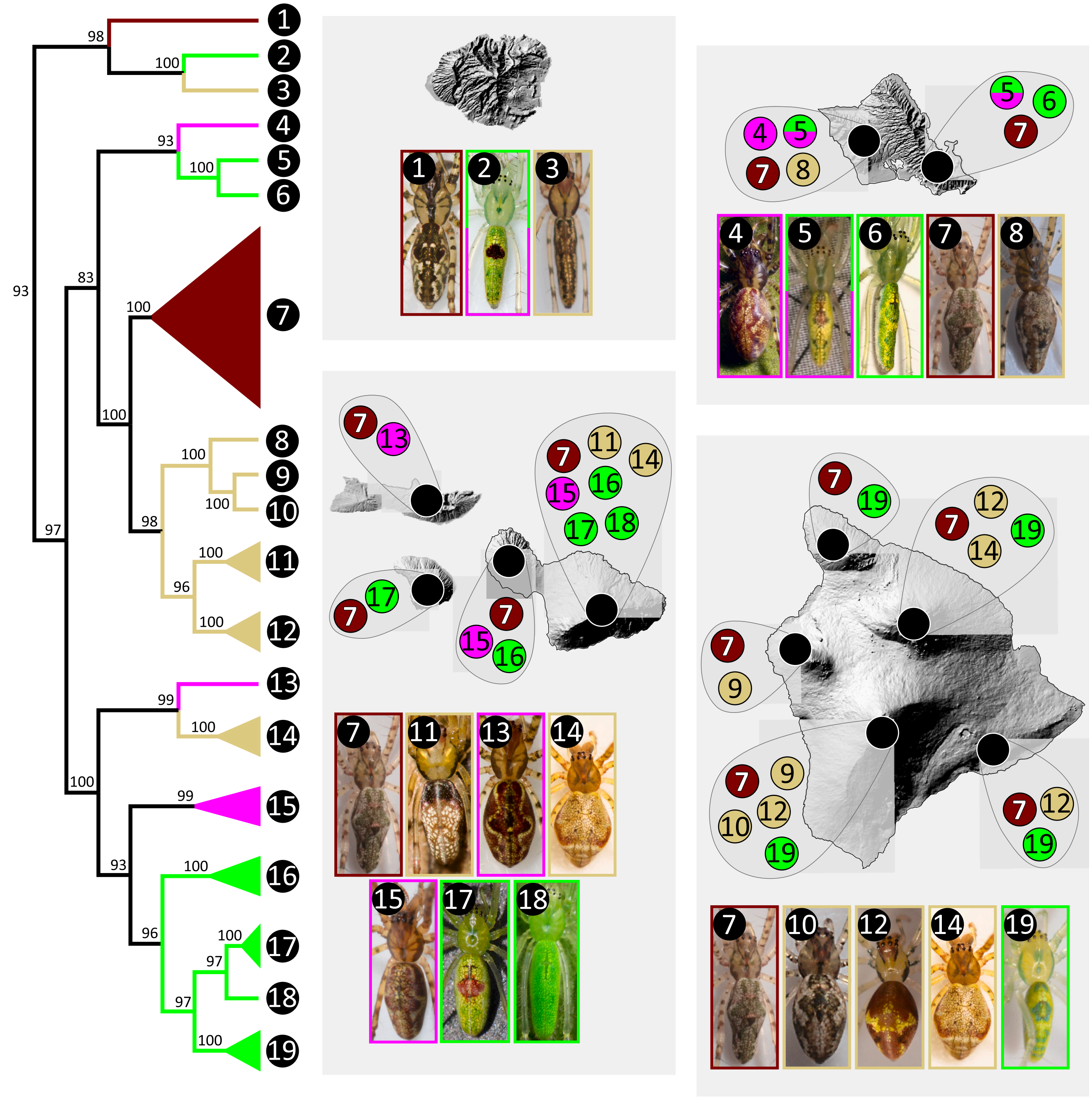

Fig. 6. Scenario 3 – Character displacement in the Spiny Leg clade. Maximum likelihood phylogeny of the Spiny Leg clade, which shows evidence for diversification driven in part by character displacement. Branch colors correspond to the Spiny Leg ecomorphs (Gillespie 2004): dark brown = Large Brown ecomorph, green = Green ecomorph, light brown = Small Brown ecomorph, purple = Maroon ecomorph. Species ranges are shown at the scale of the volcano(es) on which the species occur, indicated by numbers on the map: 1 = *T. pilosa*, 2 = *T. kauaiensis*, 3 = *T. mohihi*, 4 = *T. perreirai*, 5 = *T. polychromata*, 6 = *T. tantalus*, 7 = *T. quasimodo*, 8 = *T. kukuiki*, 9 = *T. kukuhaa* (not pictured), 10 = *T. obscura*, 11 = *T. kikokiko*, 12 = *T. anuenue*, 13 = *T. kamakou* from Molokai, 14 = *T. restricta*, 15 = *T. kamakou* from Maui, 16 = *T. macracantha* (not pictured), 17 = *T. brevignatha* from Maui, 18 = *T. waikamoi*, 19 = *T. brevignatha* from the Big Island. Note that *T. kauaiensis* and *T. polychromata* both exhibit color polymorphism, such that individuals of these species can fall into either the Green or the Maroon ecomorph (Brewer et al. 2015). Bootstrap support values are shown to the left of each node. Relief map of Hawaii obtained from EROS Data Center, National Atlas of the United States. Spider photos by George Roderick, Rosemary Gillespie and Susan Kennedy. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

way, the single species *T. quasimodo* (Fig. 4B) has colonized every island except Kauai and occupies the same niche – living on tree bark – throughout its distribution, yet it inhabits a wide range of environmental conditions (from ca. 450 m elevation to sites just above the inversion layer, ca. 2200 m (Cao et al., 2007), and from mesic to wet forests).

Similar diversification linked to isolation and niche conservatism has been found across many arthropod radiations in the Hawaiian islands (Lim et al., 2021). In several lineages, ecological shifts appear to have occurred on the oldest island of Kauai only, presumably at a time when it was the only high island of the chain (Price & Clague, 2002), and hence supremely isolated; subsequent diversification has occurred between islands, but with niches or environments conserved. Notable examples

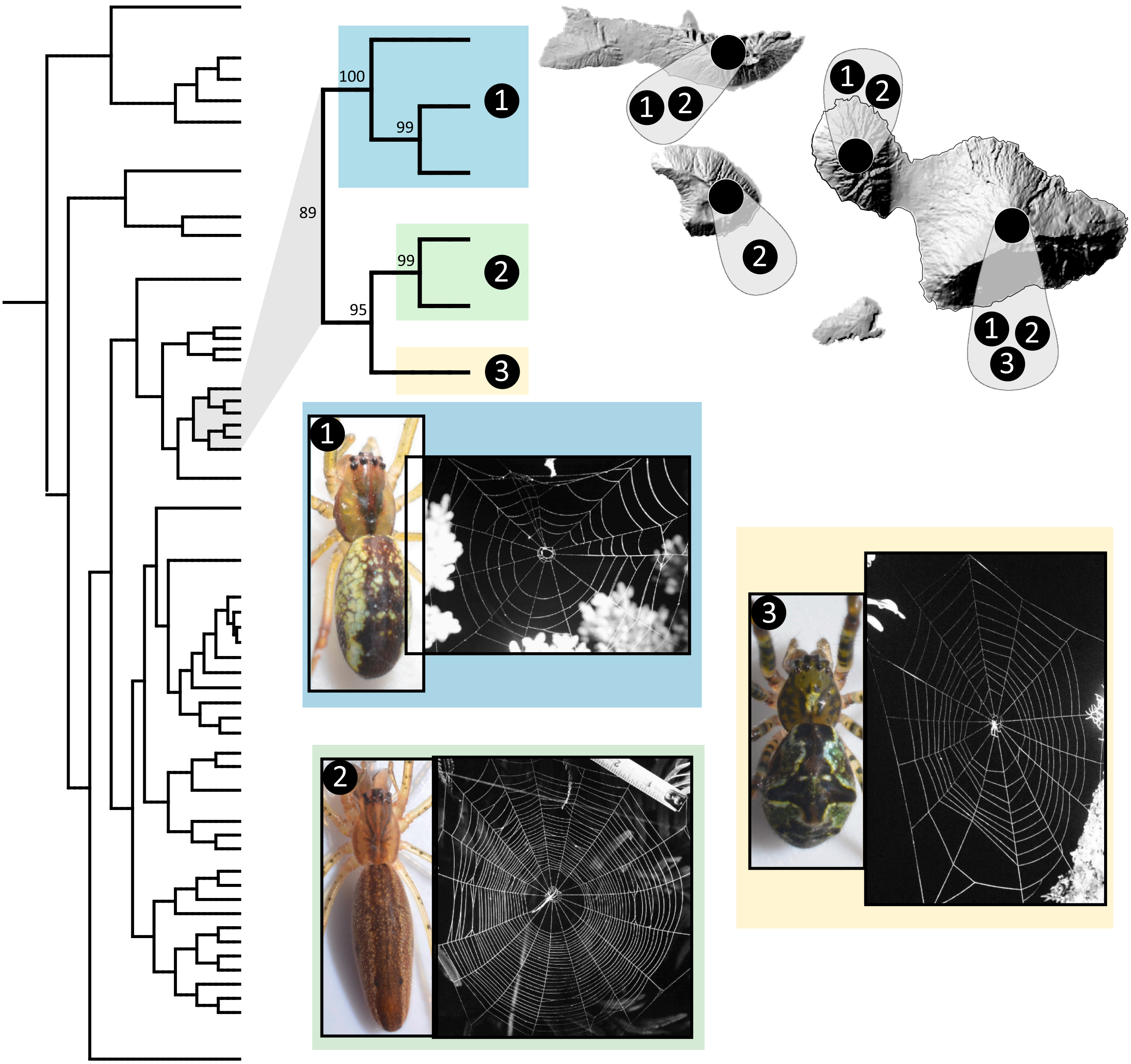

Fig. 7. Scenario 3 – Character displacement in web-builders. The Eastern Forest clade, which displays evidence for diversification driven in part by character displacement, showing its position within the web-building clade (Maximum Likelihood phylogeny). Species ranges are shown at the scale of the volcano(es) on which the species occur, indicated by numbers on the map: 1 / blue = *T. eurychasma*, 2 / green = *T. stelarobusta*, 3 / yellow = *T. trituberculata*. Although they are sisters, each of these species shows pronounced differences from the others in terms of morphology and web structure. Bootstrap support values are shown to the left of each node. Relief map of Hawaii obtained from EROS Data Center, National Atlas of the United States. Spider and web photos by Susan Kennedy and Joanne Clavel. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

are found in flies (Magnacca & Price, 2015), leafhoppers (Bennett & O'Grady, 2013) and crab spiders (Garb & Gillespie, 2009).

3.4. Scenario 2: Adaptation to novel environments

Other lineages within the Hawaiian *Tetragnatha* appear to show marked morphological and ecological shifts that are linked to moving into, and adapting to, new habitat types. This is particularly striking in the web-builders *T. acuta*, *T. albida* and *T. kea*. All of these species share a distinctive morphology, with a high, humped abdomen that comes to a point in the center, though *T. acuta* tends to be dark or black in color, while *T. albida* and *T. kea* are entirely white or white-green (Gillespie, 1994; Fig. 5). *T. acuta* has been described from both Maui Nui and the Big Island, but our topology suggests that these two populations actually represent separate species. Maui Nui *T. acuta* is found most commonly in open wet forest – bogs and scrubland at the edge of the inversion layer (Gillespie, 1994). It is most closely related to *T. albida*, which is limited to the dry forest of East Maui (Auwahi), where the white color provides camouflage against the dense covering of *Usnea* lichens. These lichens tend to dominate the forests in areas that are not wet enough to sustain high abundances of mosses, yet have sufficient moisture, often through cloud alone, to support the white / green fruticose lichens (Smith, 1995). In a similar manner, Big Island *T. acuta* is found most commonly on open lava that separates patches of wet forest. It is most closely related to *T. kea*, which occurs at highest abundances in relatively dry areas above the inversion layer (high elevation, ca. 2200 m) on Mauna Kea and in the dry forest of Puu Waawaa, again associated with dense covering of the fruticose lichens. Two additional species – *T. maka* on Kauai and *T.* sp. "Pointed Abdomen" on Oahu – share the distinctive abdomen shape of *T. acuta*, *T. albida* and *T. kea*. Interestingly, *T. maka* and *T.* sp. "Pointed Abdomen" are also associated with habitats that are inhospitable to most other Hawaiian *Tetragnatha*: exposed, windswept ridges and cliff edges. Adaptive radiation associated with shifts to novel habitats has been well documented in other groups, in particular in fish, where there has been repeated evolution of benthic and limnetic forms among both sticklebacks (Bay et al., 2017; Peichel & Marques, 2017) and cichlids (Recknagel et al., 2014). Similarly, lineages can undergo repeated evolution of locally adapted ecotypes in response to elevational gradients, as in Galápagos wolf spiders (De Busschere et al., 2010), or when transitioning to a troglobitic lifestyle, as in Hawaiian cixiid planthoppers (Hoch, 1997).

3.5. Scenario 3: Character displacement

Still another phenomenon found in the Hawaiian *Tetragnatha* radiation is character displacement: pronounced morphological and ecological divergence driven by competition between ecologically similar yet reproductively isolated taxa within a given area. This pattern has been described extensively in the Spiny Leg clade, where diversification has been linked to the repeated evolution of almost identical sets of ecomorphs (Gillespie, 2004). Thus, there are 3–4 co-occurring species in any given wet forest habitat, representing each of the different ecomorphs – "large brown" associated with tree bark as a resting substrate, "small brown" found primarily on twigs, "green" associated with leaves, and "maroon" associated with mosses and lichens (Fig. 6). In this lineage, it appears that initial genetic divergence and reproductive isolation occur in allopatry (Cotoras et al., 2018), e.g., in forest patches separated by lava flows. Subsequently, the daughter lineages come into secondary sympatry, for example when forest re-growth connects previously isolated patches, and are subjected to strong competition which selects for niche divergence. Thus, the primary factor allowing the co-occurrence of ecologically similar taxa involves no adaptation to novel environments, but rather seems to be driven by character displacement mediated by competition (Gillespie et al., 2020).

The repeated evolution of discrete ecomorphs has been well documented in other radiations, most notably in *Anolis* lizards (Glor et al., 2003; Langerhans et al., 2006) and *Ariamnes* spiders (Gillespie et al., 2018). In each situation, it appears that character displacement is the driving force for niche divergence (Gillespie et al., 2020). However, although ecomorphs demonstrate this phenomenon, divergence between sister taxa can also occur along less obvious ecological axes while still allowing co-occurrence. For example, among web-building *Tetragnatha*, co-occurring species show marked divergence in their web structure, while the same stereotyped web forms evolve convergently in other species at other sites (Blackledge & Gillespie, 2004). This is most striking in the web-builders *T. stelarobusta*, *T. trituberculata* and *T. eurychasma* (Fig. 7), which overlap on the slopes of Mt. Haleakalā in East Maui. These three species are each other's closest relatives yet show markedly different web forms (Blackledge et al., 2003), which in turn are associated with different prey spectra (Kennedy et al., 2019). Compared to the Spiny Leg species mentioned above, fewer details are available regarding the circumstances of early divergence in these web-builders. Given that these three species are sympatric sisters, yet retain their separate identities without hybridizing, they may also be subject to reproductive isolation mechanisms such as the ability to recognize species-specific chemical profiles (see Adams et al, 2021).

3.6. Conclusions

Our results highlight the fact that adaptive radiation can proceed along multiple distinct evolutionary trajectories. If the "trigger" for adaptive radiation is the opening of ecological space, then it appears that the accumulation of species is a race between dispersal of a taxon into a given habitat and the adaptation of one that is already there (Roughgarden, 1972), and the "winners" of the race are not predictable, even within a radiation. In the case of the Hawaiian *Tetragnatha*, species have, in some situations, been able to expand their environmental and physiological amplitude to occupy novel environments (e.g., dry forest on the leeward side of the islands or above the inversion layer). This situation (Scenario 2 above), in which populations diverge to occupy different environments, is the one most commonly associated with adaptive radiations (Kusche et al., 2014; Rundle et al., 2000). However, this scenario is relatively uncommon in the Hawaiian *Tetragnatha* radiation, where most diversification has occurred within a single environment – the wet forest (Fig. 1; Hiller et al., 2019). Indeed, the wet forest is home to by far the highest diversity of species, with up to 11 *Tetragnatha* species co-occurring at any one site (Gillespie, 1992).

The majority of diversification in this system appears to have emerged from a combination of factors: lineages that have colonized from elsewhere and maintained their niche (Scenario 1), and species that have adapted (sometimes repeatedly) to different niches, presumably as a result of competition between reproductively isolated close relatives within a given site (Scenario 3; Cotoras et al., 2018). The latter situation alone appears to be characterized by repeated evolution of co-occurring and interacting species belonging to distinct ecomorphs (Gillespie et al., 2020). Our results indicate that the processes involved in adaptive radiation can be quite varied, leading to different outcomes for different clades within the radiation. Only by separating the processes, and focusing on commonalities of drivers under the different circumstances, will we be able to understand universal principles involved in species radiations.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Contributions

Conceptualization, S.R.K., J.Y.L., S.A.A., H.K. and R.G.G.; Methodology, J.Y.L., H.K. and R.G.G.; Formal Analysis, S.R.K.; Investigation, S.R.K., S.A.A. and H.K.; Resources, R.G.G.; Data Curation, S.R.K., S.A.A. and R.G.G.; Writing – Original Draft, S.R.K., J.Y.L., S.A.A., H.K. and R.G.G.; Visualization, S.R.K.; Funding Acquisition, S.R.K., S.A.A. and R.G.G.; Supervision, R.G.G.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2022.107564.

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