Convergent evolution of behavior in an adaptive radiation of Hawaiian web-building spiders

Todd A. Blackledge[†] and Rosemary G. Gillespie

Department of Environmental Science, Policy, and Management, Division of Insect Biology, 201 Wellman Hall, University of California, Berkeley, CA 94720

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Species in ecologically similar habitats often display patterns of divergence that are strikingly comparable, suggesting that natural selection can lead to predictable evolutionary change in communities. However, the relative importance of selection as an agent mediating *in situ* diversification, versus dispersal between habitats, cannot be addressed without knowledge of phylogenetic history. We used an adaptive radiation of spiders within the Hawaiian Islands to test the prediction that species of spiders on different islands would independently evolve webs with similar architectures. *Tetragnatha* spiders are the only nocturnal orb-weaving spiders endemic to the Hawaiian archipelago, and multiple species of orb-weaving *Tetragnatha* co-occur within mesic and wet forest habitats on each of the main islands. Therefore, comparison of web architectures spun by spiders on different islands allowed study of replicated evolutionary events of past behavioral diversification. We found that species within each island construct webs with architectures that differ from one another. However, pairs of species on different islands, "ethotypes," share remarkable similarities in web architectures. Phylogenetic analysis demonstrated that the species comprising these ethotypes evolved independent of one another. Our study illustrates the high degree of predictability that can be exhibited by the evolutionary diversification of complex behaviors. However, not all web architectures were shared between islands, demonstrating that unique effects also have played an important role in the historical diversification of behavior.

Hawaii | natural selection | orb web | speciation | Tetragnathidae

Natural selection is a fundamental source of evolutionary diversification (1) and a mechanism for speciation (2, 3). Organisms in comparable habitats often display strikingly similar patterns of divergence, suggesting that natural selection can lead to predictable evolutionary change (4–10). However, knowledge of phylogenetic history is necessary to assess the relative importance of selection as an agent mediating *in situ* diversification versus dispersal between habitats. Adaptive radiations within the Hawaiian archipelago provide ideal test systems for these two mechanisms because the different islands present a chronological series of replicated communities, with repeated bouts of evolutionary diversification of lineages occurring within each island and dispersal occurring between islands (11, 12).

Orb-weaving spiders in the genus *Tetragnatha* are found in most terrestrial ecosystems throughout the world but are relatively homogeneous in their behaviors (13, 14). In contrast, Hawaiian *Tetragnatha* have undergone a series of adaptive radiations within the archipelago, resulting in two closely related lineages of spiders (15, 16). The "spiny leg" clade has lost the use of webs in prey capture, whereas a second radiation of ≈35 species have retained their web-building behaviors and constitute the lineage on which we focus here. Species in the genus *Tetragnatha* are the only nocturnal orb-weaving spiders endemic to the Hawaiian Islands, and two to four locally common species typically build webs in close proximity to one another in most habitats (17, 18). The architectures of webs constructed by spiders are quantifiable products of a complex series of behaviors such that differences in web shape can indicate differences in how spiders are using resources (17, 19–21). We assess the relative predictability of behavioral evolution within the adaptive radiation of Hawaiian orb-weaving *Tetragnatha* by comparing the web architectures of species, both within islands and among communities on different islands, and by examining the historical diversification of those behaviors.

Materials and Methods

Focal Localities. We studied spiders in focal localities on three different islands, each of which consisted of mature mesic to wet forest vegetation but varied in age according to island (22): Ka'ala Natural Areas Reserve, O'ahu (≈3.7 million years old; 1,200-m elevation); Waikamoi Nature Conservancy Preserve, Maui (≈1.3 million years old; 1,400- to 1,800-m elevation); and Pu'u Maka'ala Natural Areas Reserve, Hawai'i Island (≈0.7 million years old; 1,250-m elevation). Three species of *Tetragnatha* [*Tetragnatha limu* and two undescribed morphospecies ("emerald ovoid" and "eurylike")] commonly constructed webs in the Ka'ala focal locality; four species of *Tetragnatha* (*Tetragnatha acuta*, *Tetragnatha eurychasma*, *Tetragnatha filiciphilia*, and *Tetragnatha stelarobusta*) commonly constructed webs in the Waikamoi locality; and two species of *Tetragnatha* (*Tetragnatha hawaiensis* and *Tetragnatha perkinsi*) commonly constructed webs in the Pu'u Maka'ala locality. Additional web-building species can sometimes be found in each of these localities but were very rare in comparison with the common species (only one to three individuals were located in any habitat), so they were not included in the study.

Analysis of Web Architectures. Web architectures of spiders were recorded in the field by using a Sony PC110 digital video camera (17). Spiders were surveyed in the summers of 2000 and 2001 for a total of 4–10 nights per locality. Spider webs were located through visual searches, and we included all adult and penultimate female *Tetragnatha* spiders that had intact webs (10–28 individuals per species).

We analyzed web images using the SCION IMAGE program [ported from NIH IMAGE for the Macintosh by Scion (Frederick, MD)] on a Microsoft Windows computer (17). For each web, we counted the number of non-sticky radial support threads (Fig. 1*A*) and measured directly the total web area as delimited by the outermost spiral of sticky capture silk (Fig. 1*B*). We used the formula of Heiling *et al.* (23) to calculate the total length of capture spiral silk in webs (Fig. 1*C*). We also calculated the average mesh width between rows of the capture spiral, along the vertical axis, using the formula $0.5 \times [C_u/(S_u - 1) + C_l/(S_l - 1)]$, where C_u and C_l are the lengths of the upper and lower capture areas of webs and S_u and S_l are the numbers of rows of sticky silk in the upper and lower capture areas of webs (Fig. 1*D*). Data were analyzed by using STATISTICA 6.0 (StatSoft, Tulsa,

Abbreviation: PCA, principal component analysis.

[†]To whom correspondence should be sent at the present address: Department of Biology, University of California, Riverside, CA 92521. E-mail: todd.blackledge@ucr.edu.

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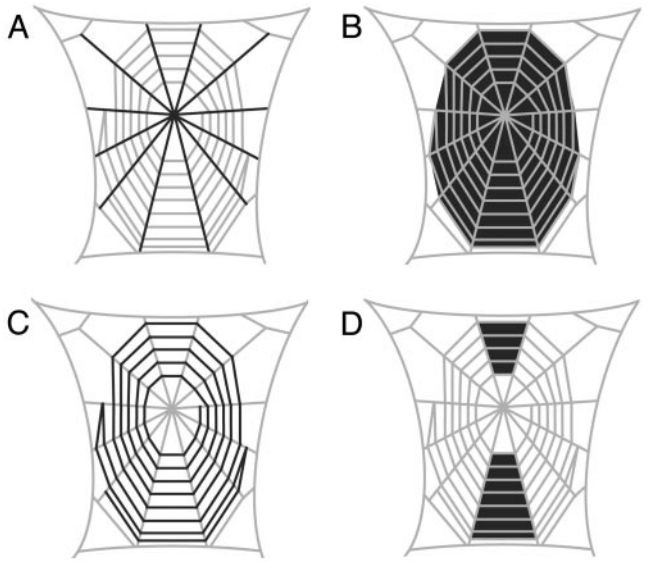

Fig. 1. Architectural features of orb webs that were quantified. (*A*) Number of radial threads. These dry silk fibers provide stiff supports for the capture surface. (*B*) Total area of the web, as delimited by the outermost row of the sticky capture spiral. (*C*) Total length of the sticky capture spiral. (*D*) Mesh width is the average distance between rows of the sticky spiral calculated along the vertical axis of the web.

OK). Each aspect of web architecture was log transformed to normalize the data, and a principal component analysis (PCA) was used to remove statistical dependence among these four variables.

Phylogenetic Analysis. To determine the historical relationships among species studied, we examined three regions of mitochondrial DNA. The mainland taxa *Tetragnatha pallescens* and *Tetragnatha laboriosa* were used as outgroups. For each species, two to five individuals were sequenced. Initial analysis indicated that all individuals within each species were monophyletic. Therefore, because the behavioral data were analyzed at the species level, relationships between taxa were examined by using the consensus sequence for each species, with differences between individuals coded as polymorphisms. The three regions of mitochondrial genes used were (*i*) a 768-bp region of cytochrome oxidase subunit I, (*ii*) a 508-bp region of the 16S rDNA gene, and (*iii*) a 205-bp region of the12S rDNA gene (see refs. 10 and 24 for details). The combined mitochondrial DNA sequence data set consisted of a total of 1,481 bases.

Phylogenetic hypotheses were reconstructed from the sequence data by using maximum parsimony, Bayesian inference of likelihood, and maximum likelihood methodologies. Maximum parsimony was implemented in PAUP* (25) by using full heuristic searches. Branch support was measured by using 200 bootstrap pseudoreplicates with uninformative characters removed (26). Bayesian inference of likelihood was implemented by using MRBAYES version 3.0b4 (27). Six chains were run simultaneously for 1,000,000 generations each, and topologies were sampled at intervals of 1,000 generations within each chain. MODELTEST 3.04 (28), which makes use of log likelihood scores to establish which of 56 models of DNA evolution best fits the data, was used to determine the model of nucleotide substitution for the Bayesian analysis. Maximum likelihood was implemented by using PAUP*. MODELTEST 3.04 was again used to determine the best-fitting of 56 models of DNA evolution. Branch support was measured by using 100 bootstrap pseudoreplicates with uninformative characters removed.

Results

The PCA of architectural features of webs resulted in two axes that described >90% of the variation within the original data set. PCA axis 1 largely described variation in sizes of webs, whereas PCA axis 2 largely described variation in the number of supporting radii and density of the capture spiral (i.e., "silk density"). Within each island, species of *Tetragnatha* constructed webs that were architecturally distinct from sympatric species (Fig. 2 *A–C*). However, comparison of the mean architectures of webs among species on different islands revealed pairs of species that overlapped with one another in web shape (Fig. 2*D*). Three pairs of species were found to belong to distinct web "ethoypes" (Fig. 2 *E–J*), which we defined as groups of species that displayed at least partial overlap in their mean web architectures (mean ± 95% SE), and whose mean web architectures were closer to one another in the principal component space than to all other species outside of those groups [e.g., a behavioral definition similar to the designation of "ecomorph" (4)].

Maximum parsimony, Bayesian inference, and maximum likelihood resulted in topologies that did not contradict one another but which did differ in levels of support for various nodes (Fig. 3). The maximum parsimony topology was fully resolved with all branches supported by bootstrap values of ≥50%, except for one node with 49% support (tree length = 643, consistency index = 0.77, homoplasy index = 0.23, retention index = 0.44, and rescaled consistency index = 0.34). MODELTEST 3.04 suggested using GTR+I+G as the best-fitting of the 56 models of nucleotide substitution tested for Bayesian inference. The Bayesian inference analysis resulted in a burn-in of 20,000 generations, with a log likelihood range, after burn-in, of −5,306.356 to −5,281.289 for the preferred topologies. The Bayesian 50% majority rules consensus of the preferred topologies did not contradict any of the relationships from the maximum parsimony analysis, although support values for clades were different. Similarly, the topology of the maximum likelihood analysis differed from that of the parsimony analysis only in the support of various nodes. With the exception of *T. hawaiensis*, which likely represents a separate introduction to the archipelago (16), species on younger islands were derived from ancestors on older islands (Fig. 4). This is a classic biogeographic pattern exhibited by many adaptive radiations within the Hawaiian Islands (11).

None of the three ethotypes were monophyletic (Fig. 4). To better test the hypothesis that each ethotype evolved only once, we generated the best tree for the data under the constraint that each ethotype had a single origin, and we compared this constrained topology to the preferred maximum parsimony topology. To accomplish this, we scored one new multistate character using each of the three ethotypes as separate character states. These character states were unordered so that each ethotype could have potentially arisen from any of the others, but the character itself was weighted by 1,000 so that each character state would have only a single origin. This technique provided a more robust test of the single origins of ethotypes than would simply constraining ethotypes to monophyly, because the latter approach would have excluded the possibility that ancestors of one ethotype could give rise to another ethotype. This tree was then used as the constraint tree to determine whether single origins of ethotypes resulted in a significant increase in length when compared with the actual data. Constraining the tree to allow each ethotype to evolve just once resulted in a significant increase in length [Templeton (Wilcoxon signed-ranks) test $z = -2.47$ and $P = 0.01$ (29); winning-sites (sign) test, $P = 0.01$ (30) for maximum parsimony; Kishino-Hasegawa test, $t = 2.50$ and $P = 0.01$ (31) for Bayesian inference and maximum likelihood].

Discussion

We found substantial differences in the web-building behaviors of different species of Hawaiian *Tetragnatha* spiders, particularly

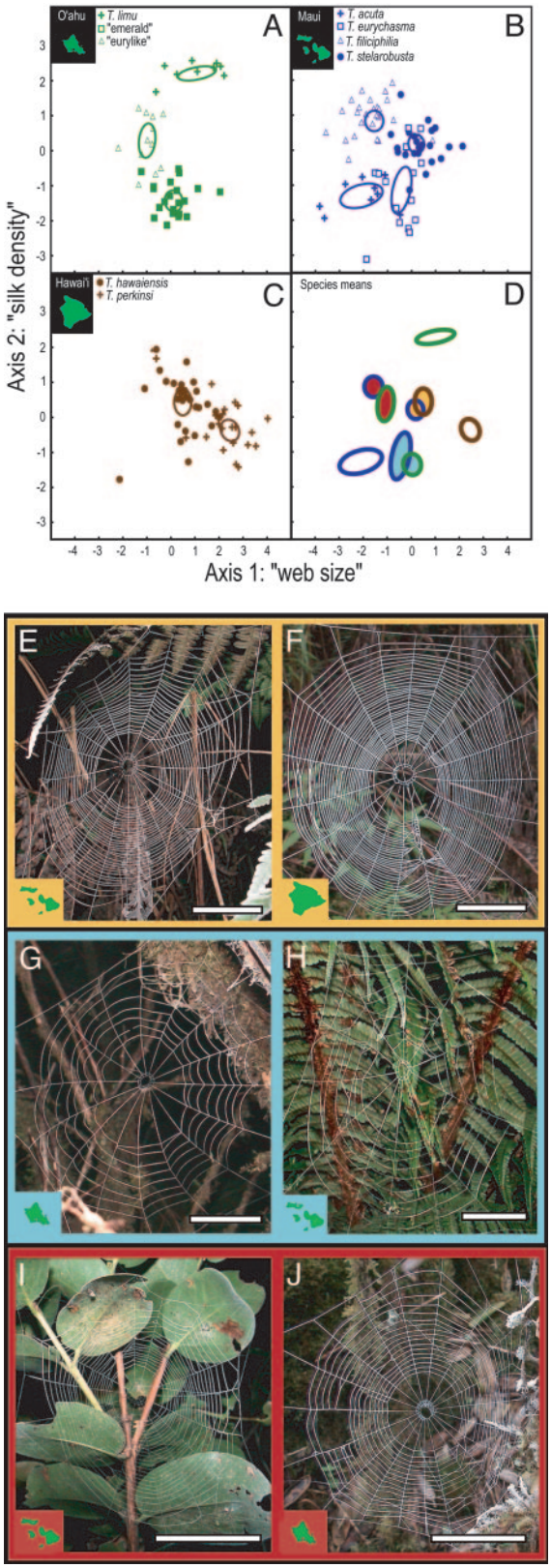

Fig. 2. Comparison of web-building behaviors among species of *Tetragnatha* on three different islands in the Hawaiian archipelago. Variation in architectures of individual spiders' webs has been summarized along two PCA axes. PCA1 largely contrasts smaller, medium, and larger webs, whereas PCA2 contrasts webs with relatively little silk (loose, open, sticky spirals and few radii) versus webs with medium and relatively dense silk (longer, tightly packed spirals of sticky silk and many radii). (*A–C*) Variation in web architecture of individual species within each island. Ellipses denote 95% confidence intervals for the mean (±SE) of species along each axis. (*D*) Comparison of species means across islands reveal three ethotypes where pairs of species construct webs that are more similar to one another than to all other species. (*E–J*) Exemplar webs showing each ethotype are bordered by the same color as in *D*. Islands are indicated by the silhouettes in the lower left corner of each picture. Medium-sized, medium-silk-density webs are constructed by *T. stelarobusta* on Maui (*E*) and *T. hawaiensis* on Hawai'i (*F*). Medium-sized, low-silk-density webs are constructed by the emerald ovoid morphospecies on O'ahu (*G*) and *T. eurychasma* on Maui (*H*). Small, medium-silk-density webs are constructed by *T. filiciphilia* on Maui (*I*) and the eurylike morphospecies on O'ahu (*J*). (Scale bar, 5 cm.)

among sympatric species (Fig. 2 *A–C*). Blackledge *et al.* (17) were the first to detail differences in web construction and prey capture within a community of Hawaiian *Tetragnatha*. Our study suggests that ecological differentiation of web construction behaviors may be widespread within the radiation of Hawaiian *Tetragnatha*. However, comparison of behaviors between species on different islands also revealed that three pairs of species constructed remarkably similar webs, which we designated as ethotypes.

Two hypotheses could explain the existence of similar ethotypes on different islands. Natural selection could have regulated how species with distinct web ethotypes dispersed between habitats, such that each ethotype evolved only once and then dispersed to other islands [i.e., "ecological assortment" (e.g., refs. 10 and 32)]. Alternatively, selection could have led to the *in situ* diversification of the behaviors among sympatric species, such that species within each ethotype evolved independently on different islands [i.e., "ecological convergence" (e.g., refs. 4 and 8)]. To differentiate between these hypotheses, we examined the historical relationships among the members of each ethotype (Fig. 4). Trees constructed under the constraint that each ethotype had a single evolutionary origin were significantly longer than the original, unconstrained topology, supporting independent origins of the behaviors of species within ethotypes.

This convergent evolution of behaviors by species within ethotypes is unlikely to result from physical constraint to a limited range of possible web architectures. Within our analysis, individual spiders filled the entire "behavioral space" delimited by the PCA (Fig. 2 *A–C*), demonstrating that it was physically possible to construct webs characterized by any combination of the two PCA axes. However, individuals were not dispersed randomly across that PCA space or we would not have found such strong differences among the mean web architectures of sympatric species. Furthermore, species with unique architectures, *T. acuta*, *T. limu*, and *T. perkinsi*, were found within each of the three focal localities (Fig. 2 *A–C*). Thus, Hawaiian *Tetragnatha* on different islands may be converging with one another in their web construction behaviors because of common selective pressures. For instance, web architecture may play a direct role in determining the effectiveness with which spiders are able to capture different types of insects, such that species of *Tetragnatha* within the same ethotype may capture similar types of prey. Members of different ethotypes within the Waikamoi focal locality tend to capture unique subsets of insect prey compared with one another and with the insects available in the environment (17), but data are lacking to test for similarities in prey capture between ethotypes on other islands. Predation risk can also play an important role in the evolution of web architecture (33). However, many common predators of mainland spiders are lacking in Hawai'i, and the most likely predators of Hawaiian *Tetragnatha*, besides other spiders, are native honeycreepers that hunt during daylight when these spiders have removed their webs. Even sexual selection could play a role in

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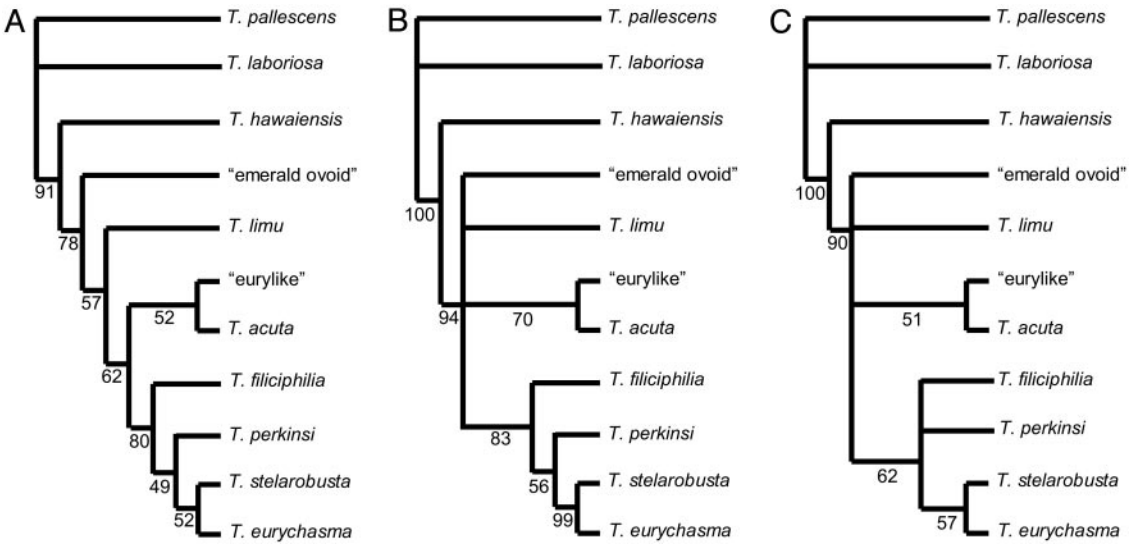

Fig. 3. Phylogenetic relationships among focal taxa and two mainland outgroups reconstructed using maximum parsimony (*A*), Bayesian inference (*B*), and maximum likelihood (*C*). The three topologies do not contradict one another and differ only in the degree of resolution of relationships. Numbers represent support values derived from bootstrap (*A*), posterior probabilities (*B*), and bootstrap analyses (*C*).

shaping the evolution of tetragnathid web architecture, because courtship and mating take place on the web.

To further investigate the pattern of evolution of web architecture within this radiation of Hawaiian spiders, we reconstructed the evolutionary change along each PCA axis. Because our analysis focused on the evolution of ethotypes as discrete character states, we chose to code the PCA axes as separate, discrete, ordered, multistate characters based on PCA factor values of less than −1.0, −1.0 to 1.0, and >1.0. These three character states roughly corresponded to small, medium, and large webs for PCA axis 1 and to low-, medium-, and high-silk-density webs for PCA axis 2. Fig. 5 illustrates the reconstruction of web size and silk density using MACCLADE 3.04 (34). A total of seven evolutionary changes in architecture were necessary to explain the existing pattern of web shape in the nine species of Hawaiian *Tetragnatha* that we studied.

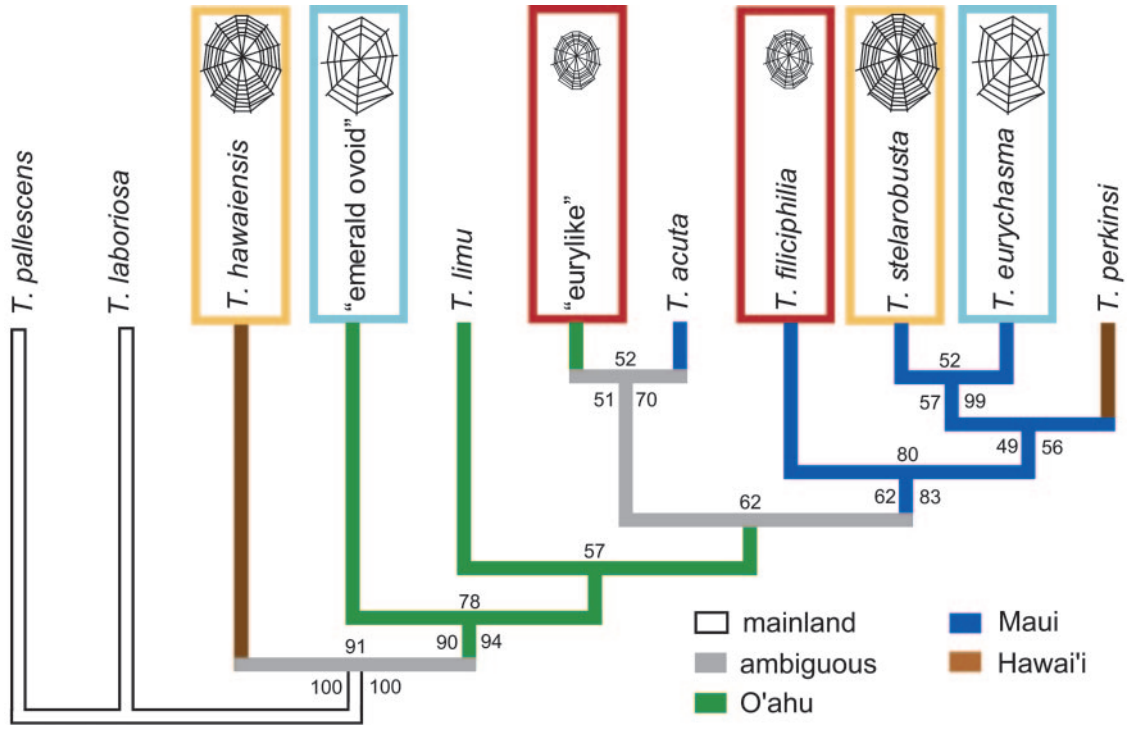

Fig. 4. Phylogenetic analysis of the historical relationships among species. Bootstrap values from the maximum parsimony analysis are presented above each node, bootstrap values from the maximum likelihood analysis are presented to the left of each node, and clade posterior probabilities from the Bayesian inference analysis are presented to the right of each internode. Colors of the branches denote different islands optimized as unordered and unpolarized character states. Web illustrations denote the three ethotypes and are identified by the same colors as in Fig. 2. Members of each ethotype arose independently of one another in at least two of the three instances.

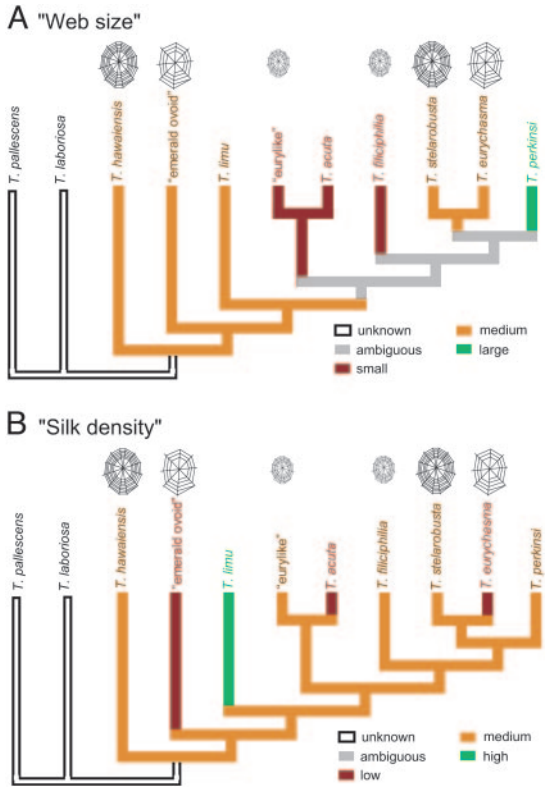

Fig. 5. Reconstruction of the evolution of web architecture. Evolutionary changes in the PCA axes in Fig. 2 *A–D* are reconstructed as separate characters with three discrete states each. Character states were defined as species means within the ranges less than −1.0, −1.0 to 1.0, and >1.0. These values corresponded to small, medium, and large webs for PCA axis 1 (*A*) and low-, medium-, and high-silk-density webs for PCA axis 2 (*B*). Character optimization was performed under the assumption that character states were ordered, but unpolarized, and optimizations were performed by using both the ACCTRAN and DELTRAN options in MACCLADE 3.04, with ambiguities coded in gray.

However, only two changes for both web size and silk density were required to explain the repeated evolution of the three ethotypes, with the additional three changes in either web size or silk density resulting in the three species with unique web architectures. This analysis suggests that medium-sized and medium-silk-density webs may represent the ancestral state for Hawaiian *Tetragnatha*. When character states were optimized such that evolutionary change was delayed relative to the root of the phylogeny (the DELTRAN option in MACCLADE 3.04) then the *T. hawaiensis* and *T. stelarobusta* ethotype could be explained by a single origin. However, optimization of web size was ambiguous within the more derived Hawaiian *Tetragnatha* (Fig. 5*A*), such that accelerating character transformations toward the phylogeny's root (the ACCTRAN option of MACCLADE 3.04) resulted in a reversal of web size from small back to medium for the *T. stelarobusta* and *T. eurychasma* clade, indicating a convergent origin of this ethotype. In this case, the small, medium-silk-density web architectures of *T. filiciphilia* and the eurylike morphospecies would instead have a monophyletic, albeit derived, origin. Regardless of these alternative optimizations, analysis of web shape as two separate characters again supports the hypothesis of convergent evolution for at least two of the three ethotypes.

Predictable evolutionary convergence among species may be a more general phenomenon than is sometimes argued, at least within adaptive radiations across island ecosystems. Radiations of both Caribbean *Anolis* lizards (4, 32) and the spiny leg clade of Hawaiian *Tetragnatha* (10) exhibit repeated patterns of morphological evolution by species on different islands. Patterns of repeatable morphological divergence are also exhibited by cichlid fish within Lake Tanganyika (7), stickleback fish in postglacial lakes (35), and even across continent-scale geographies by lineages such as bats (36) and river dolphins (37, 38). In contrast to these studies, we focused directly on behavior rather than morphology, yet we still found evidence for strong convergent evolution within the adaptive radiation of Hawaiian web-building *Tetragnatha*. This behavioral convergence is particularly striking because members of the ethotypes can be quite different from one another in size and shape, suggesting that behavioral evolution may be decoupled from morphological evolution in this system.

In our study, no ethotypes were shared between the oldest island of O'ahu and the youngest island of Hawai'i, whereas the intermediate-aged island, Maui, shared ethotypes with both the younger and older islands. This pattern is consistent with evolutionary shifts in web architecture being most predictable within a narrow time scale and could be caused by environmental changes associated with the succession-like changes in the geology of the islands as they change from initially tall, smooth volcanic cones to lower, highly eroded mountaintops (22). The small sample size of islands in our study makes it impossible to rule out that this correlation between age of islands and evolutionary similarity of communities is simply a chance effect. However, the presence of numerous adaptive radiations of other organisms within the Hawaiian Islands, as well as communities of different species of *Tetragnatha* within other habitat types on these islands, could allow for this hypothesis to be more fully tested in the future.

Our study suggests that species of Hawaiian *Tetragnatha* on different islands have independently evolved similar web-building behaviors. Thus, evolution can act in surprisingly predictable ways even upon complex behavioral traits. Future studies should focus on the functional implications of these behavioral convergences as a means to test the potential role natural selection has played in the evolutionary radiation of Hawaiian spiders.

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