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COMPARISON OF RATES OF SPECIATION IN WEB-BUILDING AND NON-WEB-BUILDING GROUPS WITHIN A HAWAIIAN SPIDER RADIATION

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ABSTRACT. The isolation of the Hawaiian archipelago has resulted in a fauna that shows high levels of endemism. I examined the role of lifestyle, as inferred from web-building versus non-web-building behavior, in dictating the rate of differentiation and species formation within a lineage of spiders in the genus *Tetragnatha* from the Hawaiian Islands. This genus comprises a group of morphologically, ecologically and behaviorally diverse taxa. Included in the radiation is a 'spiny-leg' clade which never builds webs and is relatively loosely associated with a specific habitat, and a large group of web-building species which are generally more tightly associated with a given substrate and habitat. Sequences of mitochondrial cytochrome oxidase DNA provided relative estimates of the age of a clade. Both linear and logarithmic models were used to estimate rates of speciation and the relative time required for speciation for each clade. The results showed that several small clades of web-building species have a greater rate of speciation as compared to the 'spiny-leg' clade. One explanation is that the web-building species may be capable of differentiation between more closely contiguous habitats, which would be consistent with the hypothesis that ecological differentiation promotes diversification and species formation. Possible alternative explanations for the results include differences in rates of molecular evolution, for example as a consequence of differences in metabolic activity.

The Hawaiian archipelago provides a natural laboratory for studies of speciation (Simon 1987). First, the extreme isolation of the islands has caused accentuation and acceleration of evolution in the archipelago, with numerous examples of rampant species proliferation (for reviews see Wagner & Funk 1995; Roderick & Gillespie 1998). Further, the islands are a series of volcanoes arranged within an identifiable chronological time frame (Carson & Clague 1995). The currently high islands range from Kauai, the oldest and most eroded, to Hawaii, the youngest, highest, and largest, with five separate volcanoes. Each volcanic mountain therefore shows a different stage in the evolutionary history of a clade, and allows determination of the nature of the relationship between evolutionary time and the abundance and distribution of a set of species. Radiations of spiders in the Hawaiian Islands include the genera *Tetragnatha* (as described below), *Argyrodes* (Simon 1900), *Theridion* (Simon 1900), and species in the family Thomisidae (Simon 1900; Suman 1970; J.E. Garb, this volume), among others (Gillespie et al. 1998).

I have been examining patterns of speciation (Gillespie 1991a, b, 1992a, Gillespie 1993; Gillespie & Croom 1995; Gillespie et al. 1994) and extinction (Gillespie 1992b, Gillespie & Reimer 1993) in a radiation of Hawaiian spiders in the long-jawed orb-weaving genus *Tetragnatha* (Tetragnathidae). Outside the archipelago, *Tetragnatha* is of worldwide distribution (Levi 1981), yet it is also one of the most homogeneous genera of spiders, in both morphology (elongate form [Kaston 1948]) and ecology (Caraco & Gillespie 1986; 1987a, b; Gillespie & Caraco 1987). Until 1991, information on the endemic Hawaiian tetragnathids was based on descriptions of only nine species (Karsch 1880; Simon 1900) in the genus *Tetragnatha* and one *Doryonychus*. Over the last 11 years I have collected native *Tetragnatha* in every native habitat type on all of the Hawaiian Islands. I have now described an additional 25 species of Hawaiian *Tetragnatha* (Gillespie 1991a, 1992a, 1994) and more than 60 additional new taxa have been collected, of which descriptions for many are near completion. The tetragnathid radiation spans a tremendous spectrum of colors, shapes, sizes, ecological affinities, and behaviors (Gillespie 1991a, b; 1992a, b; 1994). Many species are web-building, with striking patterns, colors and structural modifications of the abdomen that allow concealment within specific microhabitats. Some species have structural modifications which appear to allow specialization on specific prey types. One entire clade ('spiny-leg' clade) has abandoned web building, with the concomitant development of long spines on the legs and adoption of a cursorial predatory strategy (Gillespie 1991a). This clade was originally described as 12 species. Recent molecular evidence indicates that an additional six species belong in this clade.

For the 'spiny-leg' clade I have generated hypotheses of evolutionary relationships among some species (Gillespie 1993; Gillespie & Croom 1995; Gillespie et al. 1994; Gillespie et al. 1997) and among populations within widespread species (Gillespie & Roderick unpubl. data.) based on molecular and morphological characters. I have also begun to study phylogenetic relationships among groups of web-building species, in which the component species are more sedentary and tend to be more tightly associated with a given habitat (Gillespie & Croom 1995; Gillespie et al. 1997).

Here, I quantify the relationship between the lifestyle of a clade and the rate of speciation. I focus on two monophyletic lineages, the 'spiny-leg' clade and a large web-building group, because of the contrast in vagility that their species display. The majority of the web-building species have become extreme habitat specialists, while the 'spiny-leg' species have abandoned web-building to become cursorial predators and tend to be less specific in habitat preference (Gillespie & Croom 1995). I use molecular sequence data to compare patterns of speciation among web-building and non-web-building lineages on different islands. I then examine relative rates of sequence divergence to determine the extent to which lifestyle (i.e., web-building or cursorial) is associated with the rate of speciation and relative time to species formation. Because the web-building species tend to be more tied to specific habitat types, the influence of disruptive selection may be enhanced relative to non-web-building species, and new species may form more rapidly (Thoday 1972; Bush & Howard 1986).

METHODS

DNA sequences.—DNA sequence data have been generated based on part of the cytochrome oxidase subunit I (COI) mitochondrial DNA for almost all known representatives of Hawaiian *Tetragnatha*, and mitochondrial 12S ribosomal DNA sequences and allozyme data have been obtained for some representatives (Gillespie et al. 1997; R.G. Gillespie unpubl. data). For the current study I present molecular data from COI mitochondrial DNA only. A 450 base pair piece of COI was amplified for a minimum of two individuals of each species using primers C1-J-1718 and C1-N-2191 (designed by R. Harrison lab, Simon et al. 1994). Amplification was done with the following profile: 94 °C (60 sec), 48 °C (35 sec) and 72 °C (45 sec) for 40 cycles. Automated cycle sequencing was used to run and score the sequencing gels (ABI 377). Each sequence plot was inspected in Sequencher 3.1 (Gene Codes Corporation 1998). Alignments, which are straightforward for this protein-coding region, were performed by eye.

Species sampling.—The current study examines relative rates of speciation. Accordingly, I had to identify monophyletic clades, with all representatives included, prior to the analysis. For each of the clades chosen, the species group is recovered consistently by maximum parsimony and maximum likelihood analyses, and has bootstrap support of > 50% (most much higher; R.G. Gillespie unpubl. data). For the current study I obtained sequence data as follows: *'Spiny-leg' clade:* I obtained additional data from COI so as to have sequences for each of the following species, all of which have been described: *T. pilosa* Gillespie, *T. kauaiensis* Simon, *T. perreirai* Gillespie, *T. tantalus* Gillespie, *T. polychromata* Gillespie, *T. waikamoi* Gillespie, *T. brevignatha* Gillespie, *T. macracantha* Gillespie, *T. restricta* Simon, *T. kamakou* Gillespie and *T. quasimodo* Gillespie. Sequences were also obtained for an additional six representatives of the 'spiny-leg' clade which are undescribed. These 17 species encompass all known representatives of the 'spiny-leg' clade except for *T. mohihi* from Kauai, a small species that is known only from a single male (Gillespie 1991a).

Web-building species: I examined all known web-building species, most of which are undescribed. However, the analysis of speciation rates focused only on groups with $>$ 50% bootstrap support (most much higher). One clade (54% bootstrap support) is from Maui and includes three described species: *T. stelarobusta* Gillespie, *T. trituberculata* Gillespie and *T. filiciphilia* Gillespie. A second clade (89% bootstrap support) is from Oahu, and includes four undescribed species which are readily identified on the basis of morphology (gross morphology and genitalic structure, Gillespie unpubl. data) and ecology: ''Elongate Palikea'' (Waianae Mountains, mid-elevation mesic forest), ''Elongate Tantalus'' (Koolau Mountains, mid-elevation mesic forest), ''Slender Elongate'' (Waianae Mountains, low elevation dry forest) and ''Elongate Kaala'' (Waianae Mountains, high elevation wet forest). A third clade (95% bootstrap support) is found on Kauai (''Elongate Kauai''), high elevation habitats on Maui (''Elongate Maui Crater'') and Hawaii (''Elongate Mauna Kea''), and mid-elevation wet forest on Hawaii (''Elongate Hawaii Saddle'').

Phylogenetic reconstruction.—Phylogenies were reconstructed using maximum likelihood as implemented in PHYLIP (Felsenstein 1993). This method can accommodate the heavy AT bias in the nucleotide composition (approximately 65% of the bases were A or T), using base frequencies estimated from the data. Maximum parsimony (PAUP* 4.0, Swofford 1998) was also used to test the robustness of the phylogenetic reconstruction. Transversions were weighted 4× transitions, roughly approximating the greater frequency of base changes that involved transitions (4×) between closely related species. Branches having maximum length zero were collapsed to yield polytomies.

Rates of speciation.—I used both linear and logarithmic models to estimate speciation rates as described by McCune (1998). Under the linear model, which assumes a ''comb-shaped'' tree, the rate of speciation (SR_{lin}) is calculated as:

$$SR_{lin} = n/t,$$

where n is the number of known species in a monophyletic clade and t is the age of the clade. The time required for speciation (TFS_{lin}) is:

$$TFS_{lin} = t/d = t/(n-1).$$

Under the logarithmic model, which assumes a symmetrical tree, the rate of speciation (SR_{ln}) is:

$$Sr_{ln} = \ln n/\ln 2,$$

with the time required for speciation (TFS_{ln}) as:

$$TFS_{ln} = t/d = t \ln 2/\ln n.$$

RESULTS

Molecular phylogenetic analysis.—For the range of genetic distances encompassing the major radiation of Hawaiian *Tetragnatha* both transitions and transversions increased linearly when plotted against Tamura distance (Tamura 1992) suggesting that both transitions and transversions are phylogenetically informative at this level and that the data, even at third positions, are not phylogenetically saturated (Gillespie et al. 1997). For the greater distances between the major Hawaiian radiation and species in the '*T. hawaiensis*' clade (a separate introduction into Hawaii, Gillespie et al. 1994) transversions are still informative, although transitions show evidence of saturation. For the major radiation, sequences for the 450-base-pair homologous region were compared and variation was found at 204 different sites, with 111 being phylogenetically informative.

Phylogenetic relationships among representatives of the 'spiny-leg' clade of Hawaiian *Tetragnatha* based on COI sequences for each of the described species are shown in Fig. 1A. The relationships are similar to those described previously (Gillespie et al. 1997). The six recently described species all form a monophyletic group with *T. quasimodo*. However, because relationships between taxa in this clade were not resolved, these species are not included in the analysis of speciation. Relationships between species within the three selected web-building clades are shown in Fig. 1B.

Rates of speciation.—For each island group in the 'spiny-leg' clade, I summed the number of base changes from the base of the clade (marked * in Fig. 1A for each group) to the branch tip for each species in the clade. Then, for each clade, the average number of base changes for species within a clade was divided by the total number of bases to give the percent sequence divergence from the base of the clade to the present. An estimate of 2%

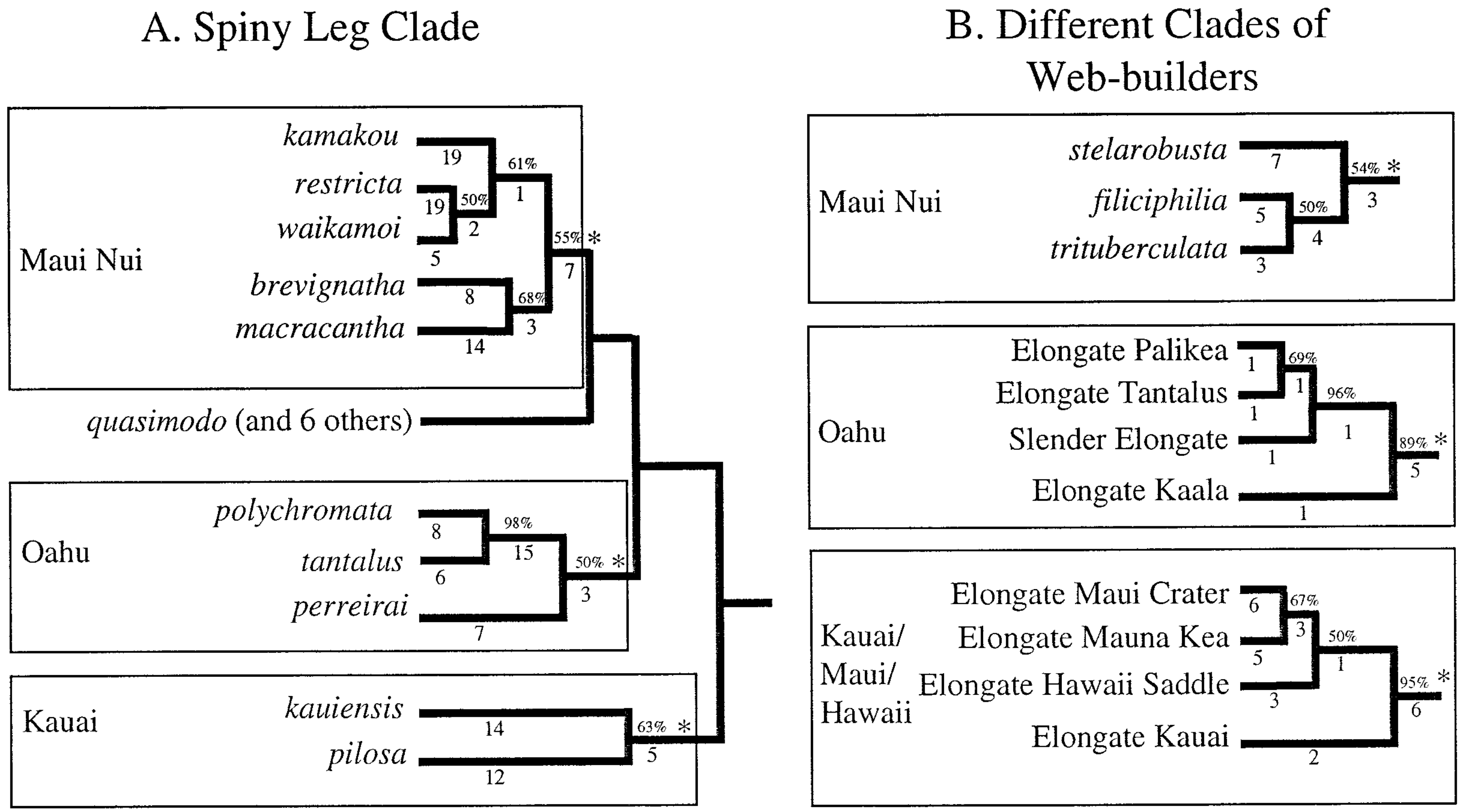

Figure 1.—Estimates of phylogeny for (A) representatives of the ‘spiny-leg’ clade of Hawaiian *Tetragnatha* (Gillespie et al. 1997) and (B) Different clades of web-building species. Numbers below branches indicate actual numbers of bases changes associated with that branch. * indicates the branch that was used as the “base” for each clade, and from which the numbers of base changes to the terminal nodes was counted.

Table 1.—Age of each clade, number of species, speciation rates, and times to speciation for: (A) the ‘spiny-leg’ clade, and (B) three different clades of web-building species, of Hawaiian *Tetragnatha.* * calculated assuming 2% sequence/10^6 years (DeSalle et al. 1987). ¶ represents divergence from Oahu.

	A			B		
Island	Kauai	Oahu	Maui	Kauai–Hawaii	Oahu	Maui
Age (myrs)	5.1	3.7	1.9	5.1–0	3.7	1.9
Number of species	2	3	5	4	4	3
Av. % seq divergence	4.00	4.45	5.03	3.16	1.61	2.37
Age of clade (myrs)*	2.00¶	2.23	2.51	1.58	0.81	1.19
SRlin	1.00	1.35	1.99	2.54	4.97	2.53
TFSlin	2.00	1.11	0.63	0.53	0.27	0.59
SRln	0.35	0.49	0.64	0.88	1.72	0.93
TFSln	2.00	1.40	1.08	0.79	0.40	0.75

base change/10^6 years was used to assess the age of a clade (DeSalle et al. 1987). This is an approximate measure, and is likely to deviate from the actual age of the clade because of the inconstancy of the molecular clock (J.H. Gillespie 1986). Accordingly, the age of the clade is used here as a relative measure only. Because the current study focuses on the comparison of two clades within the same genus in comparable habitats, base change differences between the two clades are likely to provide reliable relative estimates of differences in evolutionary rates. The age of each clade, the number of species, and both linear and logarithmic estimates of rates of speciation and relative times to speciation are given in Table 1A. Similar estimates were generated for the three clades of web-building species described above (Table 1B). Rates of speciation (both linear and logarithmic) were consistently higher for web-building species, and times to speciation were consistently lower for web-building species; these differences were all significant (Mann-Whitney *U*-test, $P < 0.05$).

DISCUSSION

The results show that the patterns of speciation relative to island age are similar in both ‘spiny-leg’ and web-building species groups: In both cases, speciation appears to have occurred largely within an island (Gillespie et al. 1997). However, the rate of species formation for the web-building clades contrasts with that of the ‘spiny-leg’ clade. In particular, differentiation between taxa within each of the web-building clades appears to occur much more rapidly. The higher rate of species formation in this clade may arise partly from their web-building habit. Based on current theories, groups that are only loosely associated with habitat types, such as the Hawaiian *Drosophila* and the cursorial ‘spiny-leg’ clade of Hawaiian *Tetragnatha*, may require longer periods of isolation in order to initiate divergence (Mayr 1963; Carson 1986; Bush & Howard 1986). However, groups comprising taxa with more rigorous ecological associations could diverge more rapidly between contiguous habitats through the action of forces such as disruptive selection (Rausher 1984; Rosenzweig 1990). Spiders that build webs tend to demonstrate stronger habitat affinities than cursorial species (Gillespie & Croom 1995), and consequently may be capable of differentiating more rapidly.

There are alternative explanations that might account for the differences in relative rates of species formation between the ‘spiny-leg’ and web-building species. In particular, both metabolic rate and generation time are known to affect rates of molecular evolution (Martin & Palumbi 1993): Higher metabolic rate and shorter generation time cause acceleration of molecular evolution. Although we have no evidence to suggest differences in generation time between the ‘spiny-leg’ and web-building species, it may be that the metabolic rate is higher among representatives of the more active ‘spiny-leg’ clade.

The age of the islands can be compared with estimates of the ages of the different clades based on sequence divergence for the 'spiny-leg' clade, assuming a constant substitution rate of 2% per million years (DeSalle et al. 1987; Juan et al. 1996) (Table 1A). The Kauai and Oahu clades might be expected to match the age of Oahu (3.7 myrs), as the formation of this island would have allowed divergence to be initiated. However, these two clades are considerably younger (2.0 and 2.2 myrs), suggesting perhaps that colonization and divergence started well after the formation of Oahu, or that molecular evolution is more rapid than that of other arthropod taxa for which calibrations have been made. On the other hand, divergence of the Maui species in the 'spiny-leg' clade are considerably older than the oldest of the islands in the Maui Nui complex (Molokai, Maui, Lanai and Kahoolawe). This result suggests that divergence of the Maui 'spiny-leg' clade was initiated prior to the colonization of Maui. Alternatively, rates of evolution may vary more than expected, and provide only very poor estimates of the age of a clade (J.H. Gillespie 1986; Kambhampati & Rai 1991). Within a lineage, acceleration in rates of sequence divergence may be associated with the formation of new species (Carson & Templeton 1984). Accordingly, the greater apparent age of the Maui species may be merely a reflection of the greater number of species.

Whether or not the DNA sequences provide an indication of actual age of a clade does not affect the major conclusions of this study. Here, the estimates of sequence divergence are used as a relative measure only, to compare groups of spiders that differ in lifestyle (web-building or non-web-building). The results suggest that there are much smaller genetic distances involved in species formation in web-building as compared to non-web-building species of Hawaiian *Tetragnatha*. It appears, therefore, that lifestyle, as indicated by the web-building habit, dictates in part the rate at which differentiation and divergence can occur within a lineage.

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