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AN ADAPTIVE RADIATION OF HAWAIIAN THOMISIDAE: BIOGEOGRAPHIC AND GENETIC EVIDENCE

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ABSTRACT. The Hawaiian Thomisidae are noted for being extremely species rich, as well as diverse in morphology and ecology. This exceptional diversity led early systematists to place the species into several genera with cosmopolitan distributions. It has been recently suggested that these species compose a single large adaptive radiation. Species-area relationships for all thomisid species and for *Misumenops* F.O. Pickard-Cambridge 1900 (Thomisidae) species for various island areas were generated. Further, a phylogenetic hypothesis was constructed based on genetic distances between the Hawaiian thomisids and various outgroups using a 450 bp region of the mitochondrial cytochrome oxidase I (COI) gene to test for close genetic relationships. Despite the extraordinary isolation of the Hawaiian islands, the numbers of *Misumenops* and total thomisid species were found to be significantly higher than predicted for an island system of its size. Phylogenetic analysis of COI suggests the Hawaiian thomisids are more closely related to each other than to representatives of genera to which they have been previously assigned. These results support the existence of a Hawaiian thomisid adaptive radiation, and merit further investigation using comparative methods.

The biota of the Hawaiian archipelago is characterized by a number of large species radiations. These radiations result from the extreme geographic isolation and topographical diversity of the islands (Carson & Clague 1995). Natural colonization of the archipelago has been largely limited to organisms having exceptional dispersal capabilities, and those species that were sucessful colonizers experienced total genetic isolation from their source populations. Founders that made the long journey frequently underwent rapid evolution due to drift and selection in extremely small populations (Carson 1994). The Hawaiian Islands themselves are volcanic, formed at a ‘‘hot spot’’ connected to the Earth’s core. As the Pacific tectonic plate continually rolls northwestward over the hot spot, new islands are formed. Consequently, they are arranged in chronological order. After initial colonization of the archipelago, it appears that taxa have frequently progressed down the island chain in a step-wise manner, resulting in repeated founder events as each new island is colonized (Wagner & Funk 1995). This unique combination of biogeographical events may explain some of the unusual aspects of the composition of the Hawaiian biota. For example, Hawaii’s terrestrial biota is considered depauperate at higher taxonomic levels due to its extreme isolation (Howarth & Mull 1992). However, the islands are exceptionally diverse at the species level, due to extensive autochthonous speciation. The diversity of Hawaiian spiders closely follows this pattern.

Of the 105 known spider families (Coddington & Levi 1991), only 10 occur naturally in the Hawaiian Islands, and many of these are represented by a single genus (Simon 1900; Suman 1964). Some of these genera have undergone extensive speciation (Gillespie *et al.* 1998). In particular, native species of the genera *Tetragnatha* Latreille 1831 (Tetragnathidae), *Argyrodes* Simon 1864 and *Theridon* Walckenaer 1805 (Theridiidae), *Misumenops* F.O. Pickard-Cambridge 1900 (Thomisidae), *Sandalodes* Keyserling 1883 (Salticidae) and *Cyclosa* Menge 1866 (Araneidae) are known to be unusually diverse and most, if not all, are endemic to the Hawaiian Islands (Table 1). It is likely that many more species within these groups remain to be discovered. However, because many species have extremely small ranges of endemism, rapid degradation of natural areas along with increasing numbers of alien species, make these spiders exceptionally vulnerable to extinction (Gillespie & Reimer 1993).

The family Thomisidae attracted attention early in the study of Hawaiian biology. R.C.L.

Table 1.—Species radiations of Hawaiian spiders.

Family	Genus	Native species	% Considered endemic	Reference
Tetragnathidae	*Tetragnatha*	>52	100	Gillespie *et al.* (1998)
Therididae	*Theridion*	>13	100	Simon (1900)
Therididae	*Argyrodes*	>30	100	Gillespie *et al.* (1998)
Thomisidae	*Misumenops*	17	100	Suman (1970)
Araenidae	*Cyclosa*	>7	100	Simon (1900)
Salticidae	*Sandalodes*	>9	88	Simon (1900)

Perkins, a pioneering Hawaiian naturalist and collector for the *Fauna Hawaiiensis* spent little time in the pursuit of spiders. However, he recognized that ‘‘the Thomisidae are probably the most interesting and important group in the Hawaiian spiders’’ (Perkins 1913). His collections were examined by Eugene Simon (1900), who described 14 new species and a new genus of Thomisidae. These 14 species were placed in the cosmopolitan genera *Misumena* Latreille 1804, *Diaea* Thorell 1869 and *Synaema* Fabricius 1775 as well as the new endemic genus *Mecaphesa* Simon 1900. In a subsequent revision of Hawaiian Thomisidae, Suman (1970) noted that the genitalia of the Hawaiian *Diaea* was extremely similar to that of the Hawaiian *Misumena* and these two genera were likely the same. Further, examination of the setation and eyes of Hawaiian spiders previously assigned to *Diaea* and *Misumena* revealed that they were actually more similar to representatives of *Misumenops,* a cosmopolitan genus, than to other representatives of *Diaea* and *Misumena*. Accordingly, representatives of *Diaea* and *Misumena* were placed in *Misumenops*. Suman considered the endemic *Mecaphesa* to be related to the genus *Ozyptila* Simon 1964, and further suggested that that all of the Hawaiian thomisids were likely derived from three separate colonizers, one that gave rise to the Hawaiian *Misumenops,* one that gave rise to the endemic genus *Mecaphesa,* and one that gave rise to the Hawaiian *Synaema* (Suman 1970). Suman’s revision also resulted in the recognition of several new species, for a total of 21 described species, 17 of which are in the genus *Misumenops*.

Recently, Lehtinen (1993) has suggested that all Hawaiian thomisids comprise an adaptive radiation generated from a single founder, one in which extensive adaptive morphological evolution has played a significant role. Examples of explosive adaptive radiations in island archipelagos illustrate how closely related species often display great morphological diversity (Grant & Grant 1989). This phenomenon is well documented in other Hawaiian spiders (Gillespie 1994; Gillespie *et al.* 1997), and other Hawaiian taxa (Roderick & Gillespie 1998). Consequently, if the Hawaiian thomisids constitute an adaptive radiation, they may represent another exceptional opportunity for understanding evolutionary and ecological mechanisms that generate rapid diversification.

In this study I examine the species richness of Hawaiian thomisids in a biogeographical framework. Classical biogeographical theory (MacArthur & Wilson 1967) states that island species diversity is a balance between immigration and extinction. What determines the rate of immigration and extinction depends largely on the size of an island and its distance from a source. In this context one would predict that the isolation and small size of Hawaii would result in a species poor fauna. Here I examine the species-area relationship for Hawaiian thomisids at the family and generic levels in comparison to other island areas. Also, I conduct a phylogenetic analysis of Hawaiian thomisid species and representatives of the genera to which they have been assigned historically, in order to provide corroborative evidence for a within-archipelago radiation.

METHODS

Biogeographical analysis.—A species-area curve was generated for both total thomisid species and for species in the genus *Misumenops*. Data for the distribution of thomisids and *Misumenops* species have been assessed in

Table 2.—Species used in sampling of a 450 bp region of mitochondrial cytochrome oxidase I.

Species	Collection locality
Misumenops anguliventris Simon 1900	Kauai, Oahu, Maui, Hawaii Island
Misumenops cavatus Suman 1970	Hawaii
Misumenops discretus Suman 1970	Kauai
Misumenops editus Suman 1970	Oahu
Misumenops facundus Suman 1970	Hawaii Island
Misumenops insulanus Keyserling 1890	Oahu
Misumenops imbricatus Suman 1970	Oahu, Maui
Misumenops junctus Suman 1970	Molokai
Misumenops nigrofrenatus Simon 1900	Kauai, Oahu, Hawaii Island
Misumenops vitellinus Simon 1900	Kauai, Oahu, Maui, Hawaii Island
Mecaphesa perkinsi Simon 1900	Oahu
Mecaphesa semispinosa Simon 1900	Oahu
Synaema naevigerum Simon 1900	Maui
Synaema globulosum Fabricius 1775	Mt. Carmel, Israel
Xysticus sp.	Westchester, New York
Ozyptila georgiana Keyserling 1880	Westchester, New York
Misumena vatia Thorell 1870	Maryland
Misumenops asperatus Hentz 1870	Maryland
Misumenoides sp.	Indiana
Diaea sp.	New Zealand

various island systems worldwide by a number of researchers: Andaman and Nicobar (Tikader 1977), Japan (Ono 1988), Britain and Ireland (Roberts 1985), Puerto Rico (Petrunkevitch 1930), Cuba (Bryant 1940), Tonga (Marples 1959), Fiji (Marples 1957), Pitcairn Islands (Benton & Lehtinen 1995), Philippines (Barrion & Litsinger 1995), Samoa (Marples 1957), Rapa (Berland 1924), Mauritius (Simon 18975), Galapagos Islands (Banks 1902), Tahiti (Berland 1934), and Marquesas Islands (Berland 1927). In the generation of the *Misumenops* species area curve, *Diaea* species from Tonga, Fiji and Samoa were substituted for *Misumenops*, as these were likely incorrectly diagnosed and are closely related to the Hawaiian thomisids (Suman 1970). Predictions of the total expected number of thomisid species and *Misumenops* species in Hawaii were calculated based on the species-area relationships generated.

Collection and identification of Hawaiian Thomisidae.—Hawaiian thomisid species were collected from native ecosystems on Kauai, Oahu, Maui, Molokai and Hawaii Island (Table 2). Specimens were collected by beating vegetation. Sexually mature spiders were identified to species using the Bishop Museum reference collection and Suman's (1970) key. Additional outgroup species were collected from North America and Israel and others were kindly supplied by Dr. J. Robinson, Dr. G. Dodson, Cor Vink and Dr. W. Shipley.

Collection and analysis of genetic data.—DNA was extracted from the 13 Hawaiian species and 7 non-Hawaiian species listed in Table 2, using a phenol-chloroform preparation followed by ethanol precipitation (Palumbi *et al.* 1991). Voucher specimens were retained and will be deposited in the Bishop Museum, Honolulu, Hawaii. A 450 bp region of the mitochondrial gene cytochrome oxidase I (COI) was amplified for at least two individuals per species using a thermal cycler, with universal primers C1-J-1718 and C1-N-2191 (Simon *et al.* 1994). The COI gene was selected because it evolves rapidly in the third-codon position (Simon *et al.* 1994), providing sufficient variation to determine relationships between closely related species. This gene has been useful for assessing genetic diversity and phylogenetic relationships in other Hawaiian arthropods (Roderick & Gillespie 1998). PCR products were sequenced using an ABI 377 automatic sequencer and were aligned using Sequencher 3.0. Pairwise genetic distance (percent nucleotide difference between sequences) was calculated for all species using the Kimura 2-parameter correction

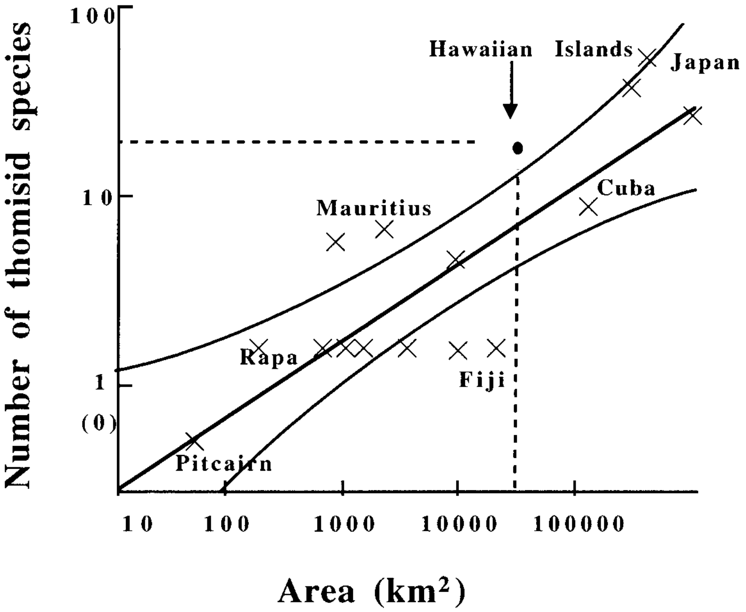

Figure 1.—Species-area relationship for total species in the family Thomisidae. Linear regression and a 95% CI on log-transformed data are shown. Equation of line is log(species) = 0.398 log(area) − 0.917, $R^2 = 0.70$, $P < 0.0001$. Expected number of species in the family Thomisidae is 7, while 21 species are observed.

for nucleotide bias (Kimura 1980), and a phylogenetic tree was constructed from these distances using a neighbor-joining algorithm in PAUP* 4.0 (Swofford 1998). The *Xysticus sp.* sequence was used to root the resulting tree.

RESULTS

Biogeographical analysis.—For the species-area in the family Thomisidae, a highly significant linear increase was found between the number of species and area of the island system (log-transformations, slope = 0.39, $R^2 = 0.70$, see Fig. 1). Perhaps not unexpectedly, Hawaii has an unusually high diversity for its size, falling just above the 95% confidence interval for the regression. The slope of the line indicates that an archipelago the area of Hawaii (28,314 sq. km), should have a total of 7 thomisid species, but 21 occur in Hawaii. The relationship for representatives of the genus *Misumenops* is quite different (Fig. 2). Whether or not Hawaii is included in the regression, there is no significant relationship between island size and number of *Misumenops* species ($R^2 = 0.49$, $P = 0.43$, for 15 islands not including Hawaii). By any measure, Hawaii appears to be an extreme outlier compared to other island systems examined. For example, Hawaii has the highest observed diversity of *Misumenops* species (17), in contrast to the 1–4 species observed in other island systems. The number of *Misumenops* species on the 15 non-Hawaiian islands appeared to be normally distributed, and assuming normality, Hawaii falls far above the 99.999th percentile of the distribution. These results suggest that although thomisid diversity in the Hawaiian islands is high in general, *Misumenops* constitutes a disproportionate amount of this diversity. Further, the restriction of 17 of Hawaii's 21 thomisids to a single genus suggests that biodiversity in this island archipelago is a function of autochthonous speciation, rather than external colonization.

Analysis of genetic data.—Construction of a distance based phylogeny from 450 bp of COI revealed that the smallest genetic distances were found between Hawaiian thomisid species (Fig. 3). Thus the Hawaiian thomisids appear to be more closely related to each other than they are to representatives of *Misumena, Misumenoides, Diaea, Synaema,* and *Ozyptila.* The Hawaiian Synaema and non-Hawaiian *S.*

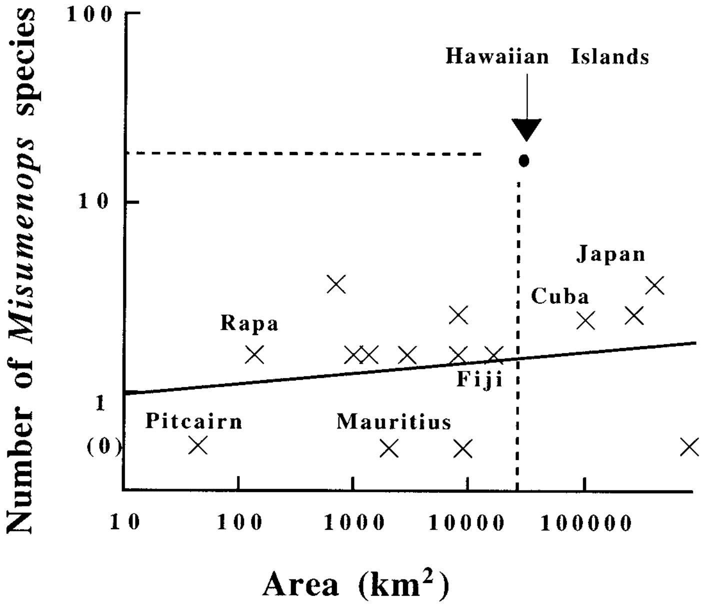

Figure 2.—Species-area relationship for species in the genus *Misumenops*, along with a simple linear regression on log-transformed data. Equation of line is log(species) = 0.054 log(area) − 0.067, $R^2 = 0.49$, P = 0.43. Expected number of Hawaiian species in the genus *Misumenops* is 0.6, while 17 species are observed.

globulosum are extremely distant while the Hawaiian *Synaema* is comparatively much more similar to the Hawaiian *Misumenops*, supporting Lehtinen's (1993) claim that the Hawaiian '*Synaema*' is not a true *Synaema*. Small genetic distances between non-Hawaiian *Misumenops asperatus* and all Hawaiian *Misumenops* species sampled suggests that the Hawaiian species are likely descended from a *Misumenops* ancestor. However, because *Misumenops asperatus* falls within the clade containing all Hawaiian thomisids, more than one colonization event is implied for the Hawaiian thomisid fauna. Sampling of many more *Misumenops* species from other geographic regions must be conducted in order to firmly establish what proportion of Hawaii's present day thomisid diversity has arisen through autochthonous species radiation.

DISCUSSION

The high diversity of *Misumenops* species in Hawaii, coupled with the short genetic distances between species, is a pattern similar to that found in other Hawaiian terrestrial arthropods that have undergone species radiation (Roderick & Gillespie 1998). While the islands are exceptionally diverse in both thomisid and *Misumenops* species, the *Misumenops* species represent nearly all of the thomisid diversity. It appears, therefore, that the number of successful colonizers is consistent with the island biogeography model. However, the model does not account for subsequent processes that occur over evolutionary time. Thus, despite few colonization events, autochthonous speciation raises the species diversity to levels higher than predicted by island area. Such a pattern might suggest that most of the available niche space for the family is being occupied by species in the genus *Misumenops*. Colonizing species may have undergone rapid ecological divergence in the absence of competition from other genera that exploit a variety of ecological strategies elsewhere.

Although Hawaiian *Misumenops* species are extremely diverse (Fig. 2), it is possible that this result may be biased by data points representing inaccurate estimates of diversity. Many of the data points used to generate the relationships between area and diversity were extracted from surveys of unequal sampling effort. Further, current revisions of these islands' spider fauna might result in different generic diagnosis of species. Consequently,

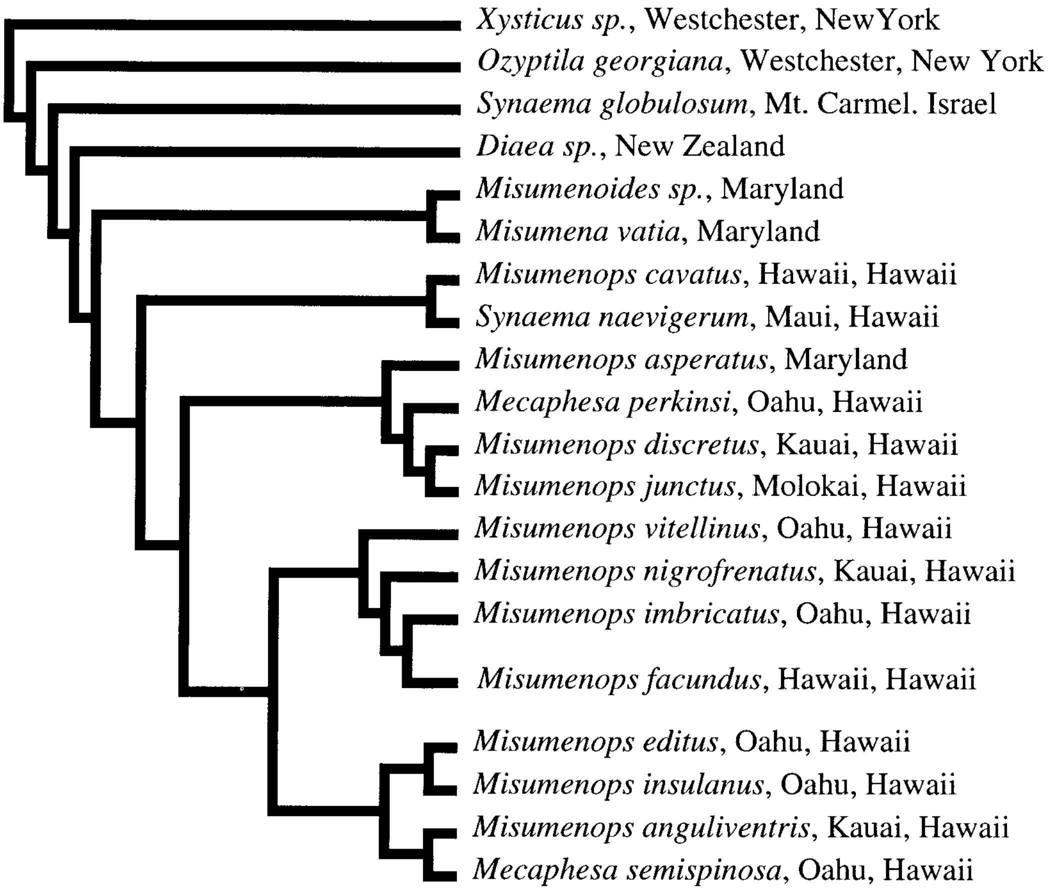

Figure 3.—A distance based phylogeny constructed from 450 bp of COI. Genetic distances were calculated using Kimura's (1980) 2-parameter corrected distance measure.

many of these points may represent over or under estimates of true diversity. It is possible that a more complete data set would reveal that Hawaii is not overly diverse in *Misumenops* or total thomisid species. However, Japan (the most thoroughly studied island group), has an area 10 times the size of Hawaii yet only twice the number of thomisid species (Ono 1988). Moreover, only three of these species are *Misumenops*. The relatively small genetic distances among Hawaiian species are consistent with the hypothesis that the high morphological and ecological diversity of Hawaiian thomisids is primarily the result of autochthonous radiation.

The Hawaiian *Misumenops* possess exceptional ecological diversity when compared to their continental congeners. Members of the family Thomisidae are well known for their employment of mimicry to ambush prey. The tribe Misumenini Simon 1895, including spiders in the genera *Misumena, Misumenoides,* and *Misumenops* (Ono 1988), are commonly known as flower spiders because they mimic the coloration of flowers on which they sit in order to capture pollinating insects (Gertsch 1979). Recent research reveals that certain species of this tribe have ecological roles as nectivores and possible pollinators (Pollard *et al.* 1995). While genetic data suggest that the Hawaiian thomisids are descendants of flower spiders, Hawaiian thomisids are extremely diverse in their substrate affinities. For example, the green and brown speckled *Misumenops editus,* endemic to the summit of Mt. Kaala, Oahu, is perfectly camouflaged amongst its primary microhabitat of moss patches. Likewise, *M. aridus* and *M. nigrofrenatus* are well hidden on their substrate of white filamentous lichen. Other species are more specific to green foliage. Many Hawaiian *Misumenops* that are ecologically separated as a result of differential substrate affinity are sympatric and may be very close relatives (Suman 1970).

In order to appreciate fully the extent of species radiation in the Hawaiian *Misumenops*, a complete phylogenetic construction is warranted. Only with a well-supported phylogenetic hypothesis can the idea of species radiation among the Hawaiian thomisids be tested rigorously. From such a hypothesis, one can determine the number of colonization events and the ecological diversity of this group. It is clear from data presented here that the Hawaiian *Misumenops* do not fit the classical model for island biogeography as they are species rich in an isolated and small island area.

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LITERATURE CITED

Banks, N. 1902. Papers from the Hopkins Stanford Galapagos Expedition. Proc. Washington Acad. Sci., 4:49–86.

Barrion, A.T. & J.A. Litsinger. 1995. Riceland spiders of South and Southeast Asia. Center for Agriculture and Bioscience, Wallingford, UK.

Benton, T.G. & P.T. Lehtinen. 1995. Biodiversity and origin of the non-flying terrestrial arthropods of Henderson Island. Bio. J. Linn. Soc., 56(1–2): 261–272.

Berland, L. 1924. Araignées de l'ile de Pâques et des îles Juan Fernandez. The Natural History of Juan Fernandez and Easter Island, 3:419–437.

Berland, L. 1927. Notes sur les Araignées recueillies aux eles Marquises par le R.P. Simén Delmas. Bull. Du Muséum National d'Histoire Naturelle, Paris, 38:366-368.

Berland, L. 1934. Les Araignées de Tahiti. Bull. Bernice P. Bishop Museum, 113:97–107.

Bryant, E.B. 1940. Cuban spiders in the Museum of Comparative Zoology. Bull. Mus. Comp. Zool., 86(7):247–554.

Carson, H.L. 1994. Genetical processes of evolution of high oceanic islands. Pp. 259–262. In A Natural History of the Hawaiian Islands. (E.A. Kay, ed.). Univ. Hawaii Press, Honolulu, Hawaii.

Carson, H.L & D.A. Clague. 1995. Geology and biogeography of the Hawaiian Islands. Pp. 14–29. In Hawaiian Biogeography, Evolution on a Hot Spot Archipelago. (W.L. Wagner & V.A. Funk, eds.), Smithsonian Institution Press, Washington, D.C.

Coddington, J.A. & H.W. Levi. 1991. Systematics and evolution of spiders (Araneae). Ann. Rev. Ecol. Syst., 22:565–592.

Gertsch, W.J. 1979. American Spiders. 2nd ed. Van Nostrand Reinhold Co., New York.

Gillespie, R.G. 1994. Hawaiian spiders of the genus *Tetragnatha*: III. *Tetragnatha acuta* clade. J. Arachnol., 22:161–168.

Gillespie, R.G., H.B. Croom & L. Hasty. 1997. Phylogenetic relationships and adaptive shifts among major clades of *Tetragnatha* spiders (Araneae: Tetragnathidae) in Hawaii. Pacific Sci., 51(4):380–394.

Gillespie, R.G. & N. Reimer. 1993. The effects of alien predation of ants (Hymenoptera: Formicidae) on the Hawaiian endemic spiders (Araneae: Tetragnathidae). Pacific Sci., 47(1):21–33.

Gillespie, R.G., M.A. Rivera & J.E. Garb. 1998. Taxonomy and phylogeography of Hawaiian Araneae. Bull. British Arachnol. Soc., 11:41–51.

Grant, B.R.& P.R. Grant. 1989. Evolutionary Dynamics of Natural Populations. Univ. Chicago Press, Chicago.

Howarth, F.G. & W.P. Mull. 1992. Hawaiian Insects and Their Kin. Univ. Hawaii Press, Honolulu.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. J. Mol. Evol. 2:87–90.

Lehtinen, P.T. 1993. Polynesian Thomisidae—A meeting of old and new world groups. Mem. Queensland Mus., 33(2):585–591.

MacArthur, R.H. & E.O. Wilson. 1967. The Theory of Island Biogeography. Monog. Population Biology 1. Princeton Univ. Press, Princeton.

Marples, B.J. 1957. Spiders from some Pacific islands II. Pacific Sci., 11:386–395.

Marples, B.J. 1959. Spiders from some Pacific islands III. The Kingdom of Tonga. Pacific Sci., 13:362–367.

Ono, H. 1988. A revisional study of the spider family Thomisidae of Japan. National Science Museum, Tokyo, Japan.

Palumbi, S.R., A. Martin, S. Romano, W.O. McMillian, L. Stice & G. Grabowski. 1991. The simple fool's guide to PCR. Dept. of Zoology, University of Hawaii, Honolulu.

Perkins, R.C.L. 1913. Introduction. Pp. 15–228. *In* Fauna Hawaiiensis, Vol. 1. (D. Sharp, ed.) Cambridge Univ. Press, Cambridge.

Petrunkevitch, A. 1930. The spiders of Puerto Rico. Trans. Connecticut Acad. Sci., 30:1–355; 31:1–191.

Pollard, S.D., W.M. Beck & G.N. Dodson. 1995. Why do male crab spiders drink nectar? Anim. Behav., 49:1443–1448.

Roberts, M.J. 1985. The Spiders of Great Britain and Ireland. Vol. 1. Atypidae–Theridiosomatidae, 229 pp. Harley Books, Colchester, UK.

Roderick, G.K. & R.G. Gillespie. 1998. Speciation and phylogeography of Hawaiian terrestrial arthropods. Mol. Ecol., 7:309–322.

Simon, C., F. Fratti, A. Beckenbach, B. Crespi, H. Liu & P. Flook. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. America., 87:651–701.

Simon, E. 1897. Arachnological records par Alluaud a l'ile Maurice. Ann. Soc. Entomol. France, 66:271–276.

Simon, E. 1900. Arachnida. Pp. 443–519. *In* Fauna Hawaiiensis, Vol. 2. (D. Sharp, ed.), Cambridge Univ. Press, Cambridge, UK.

Suman, T.W. 1964. Spiders of the Hawaiian Islands: catalog and bibliography. Pacific Insects, 6:665–687.

Suman, T.W. 1970. Spiders of the Family Thomisidae in Hawaii. Pacific Insects, 12(4):773–864.

Swofford, D.L. 1998. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Tikader, B.K. 1977. Studies of the spider fauna of Andaman and Nicobar Islands, Indian Ocean. Rec. Zool. Surv. India, 72:153–212.

Wagner, W.L. & V.A. Funk. 1995. Hawaiian Biogeography. Evolution On a Hot Spot Archipelago. Smithsonian Institution Press, Washington, D.C.

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