Report

Current Biology

Repeated Diversification of Ecomorphs in Hawaiian Stick Spiders

Highlights

- Hawaiian stick spiders show adaptive radiation with repeated evolution of ecomorphs
- This phenomenon is found in only a few adaptive radiations of island insectivores
- Camouflage against a finite set of predators and wandering habit play key roles
- Limited pathways for the development of color contribute to deterministic evolution

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In Brief

Gillespie et al. show that adaptive radiation of Hawaiian stick spiders is highly deterministic, with repeated formation of a discrete set of ecomorphs. Camouflage against a finite set of predators, reduced dispersal, and cursoriality, coupled with conserved pathways of color formation, together facilitate predictable evolutionary outcomes.

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Repeated Diversification of Ecomorphs in Hawaiian Stick Spiders

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SUMMARY

Insular adaptive radiations in which repeated bouts of diversification lead to phenotypically similar sets of taxa serve to highlight predictability in the evolutionary process [1]. However, examples of such replicated events are rare. Cross-clade comparisons of adaptive radiations are much needed to determine whether similar ecological opportunities can lead to the same outcomes. Here, we report a heretofore uncovered adaptive radiation of Hawaiian stick spiders (Theridiidae, *Ariamnes*) in which different species exhibit a set of discrete ecomorphs associated with different microhabitats. The three primary ecomorphs (gold, dark, and matte white) generally co-occur in native forest habitats. Phylogenetic reconstruction mapped onto the well-known chronosequence of the Hawaiian Islands shows both that this lineage colonized the islands only once and relatively recently (2–3 mya, when Kauai and Oahu were the only high islands in the archipelago) and that the distinct ecomorphs evolved independently multiple times following colonization of new islands. This parallel evolution of ecomorphs matches that of "spiny-leg" long-jawed spiders (Tetragnathidae, *Tetragnatha*), also in Hawaii [2]. Both lineages are free living, and both have related lineages in the Hawaiian Islands that show quite different patterns of diversification with no evidence of deterministic evolution. We argue that repeated evolution of ecomorphs results from a rugged adaptive landscape, with the few peaks associated with camouflage for these free-living taxa against the markedly low diversity of predators on isolated islands. These features, coupled with a limited genetic toolbox and reduced dispersal between islands, appear to be common to situations of repeated evolution of ecomorphs.

RESULTS AND DISCUSSION

Colonization of the World's Most Remote Archipelago and Associated Ecological Shifts

The Hawaiian archipelago, situated approximately 3,800 km from the nearest continent, is the world's most isolated landmass (Figure 1). Natural colonization of the islands is restricted to organisms possessing exceptional dispersal capabilities or those capable of the phoretic use of such organisms as vectors [3]. Paradoxically, the archipelago is also considered one of the most diverse areas in terms of species endemicity, estimated at 96% in insects and other arthropods [4] and 90% in angiosperms [5]. Islands within the Hawaiian archipelago have been continuously available for colonization for about 30 million years [6]. However, immediately preceding the formation of the current high islands, there was a period in which all islands were low, relatively small, and widely spaced. Consistent with this geological history, most lineages of extant taxa appear to have colonized the archipelago within the past 5 million years [7]; in addition, because islands are arranged chronologically [6], many lineages demonstrate an evolutionary progression from older to younger islands [8], with multiple lineages assembling on each island over a similar time frame [9]. Among those lineages that follow the island progression from older to younger islands, diversification may involve little ecological change (nonadaptive radiation) [10, 11]; alternatively, diversification may be accompanied by ecological modifications that frequently allow co-occurrence [12], the "classic" form of adaptive radiation. The current study examines the adaptive radiation of Hawaiian stick spiders in the genus *Ariamnes* (Theridiidae), represented in the islands by 11 described species [13] and at least 4 more awaiting description (Table 1). The similarity in genitalic structure, despite marked differences in ecology and morphology, suggests rapid adaptive radiation. The group is facultatively kleptoparasitic on the large webs of *Orsonwelles* (Linyphiidae) spiders [14] but are much more commonly observed as free living.

Hawaiian Stick Spiders Originated from Kleptoparasitic Ancestors

Our data confirm the placement of Hawaiian *Ariamnes* (Figures 2 and 3) within the worldwide subfamily of Theridiidae, Argyrodinae [16, 17] (Figure S1). Lineages within the Argyrodinae differ

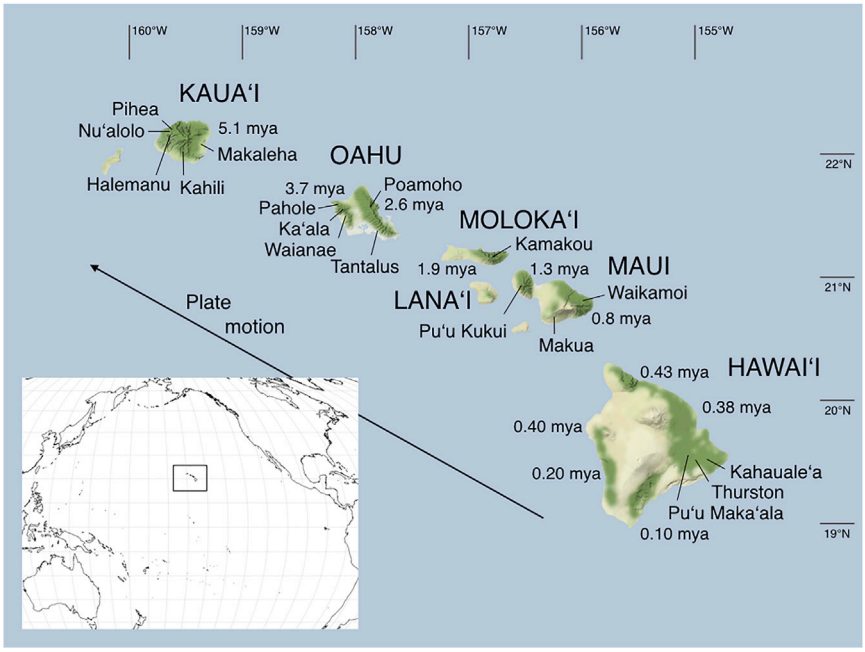

Figure 1. The Hawaiian Archipelago Chronosequence, Showing Collecting Sites for Specimens Used in the Current Study

Volcano ages are noted. Hawaii base map from http://mapstack.stamen.com.

See also Tables 1 and S1.

markedly in foraging behavior (Data S1). In particular, species in two genera, including *Ariamnes*, are known as free-living, web-invading hunters of other unrelated spiders, while those investigated in at least four other species groups form kleptoparasitic (food-robbing) associations around the webs of other spiders [18]. Even among the kleptoparasites, species groups differ in their prey-capturing techniques [19], with spiders in one group (*A. trigonum*) showing a tendency to kill their host [20], those in another (*A. cancellatus*) gleaning insects [19] but not feeding with the host, and finally those in a third (*A. argyrodes*) using all kleptoparasitic behaviors, including a specialized "feeding with the host" [21].

In the current study, the addition of a Hawaiian *Ariamnes* species (*A. corniger*) to molecular sequence data from a recent phylogenetic study of the Argyrodinae [22] shows that the Hawaiian lineage groups with some support within the "*miniaceus* clade" containing *Argyrodes miniaceus* and several other species of *Argyodes* and *Spheropistha* (Figure S1 and Data S2). Representatives of this clade outside Hawaii are all from Asia, though the limited sampling [22] precludes inference about the geographic origin of the Hawaiian radiation. However, it is evident that the Hawaiian *Ariamnes* species are not closely related to other representatives of the genus *Ariamnes*; indeed, the genus to which the Hawaiian *Ariamnes* should be assigned is not clear, given the apparent polyphyly of *Argyrodes* and uncertain placement of the genus *Spheropistha*. Species in the *mineaceus* clade are primarily kleptoparasitic and group living; because of the larger number of group-living than solitary (free-living) species, it was suggested that speciation has been more rapid among group-living species in this clade [22]. The apparent placement of the Hawaiian *Ariamnes* within this *miniaceus* clade provides an interesting contrast, as most of the Hawaiian species occur as free-living individuals, yet speciation has been very rapid.

Evolution of Ecological Diversity in Hawaiian *Ariamnes* Spiders

Adaptive radiation has been defined as the evolution of ecological diversity within a rapidly multiplying lineage [23]. The phenomenon is common on remote islands, with ecological space frequently filled by species proliferation among lineages that have limited underlying genetic diversity. In the Hawaiian Islands, the best-known spider adaptive radiation is that of the long-jawed genus *Tetragnatha* (Tetragnathidae), characterized by major ecological shifts, such as the loss of web building in the cursorial "spiny-leg" clade [24]. The current study shows that the Hawaiian *Ariamnes* have also undergone extensive adaptive radiation. Based on data from both mitochondrial (cytochrome *c* oxidase 1, CO1) and nuclear (elongation factor, EF1alpha) gene sequences, we found that relationships among species of *Ariamnes* are consistent with the progression rule described above. Support at the base of the Hawaiian radiation—between the lineages on Kauai and Oahu—is weak, likely consistent with the existence of both islands when the lineage initially colonized. Thus, while the phylogeny suggests that the lineage may have colonized the oldest island, Kauai, first (Figure 3), then hopped down the island chain in the direction of the younger islands, colonization of Oahu likely occurred very shortly after the lineage arrived. Estimates of the timing of divergence using a standard molecular clock for arthropods [25, 26] support this scenario (Figure S2), with the *Ariamnes* spiders colonizing the Hawaiian Archipelago approximately 1.7 mya (95% confidence limit 0.77–3.8 mya)—considerably more recently than the formation of the oldest island (Kauai, 5 mya) and when both Kauai and Oahu were large islands, while the Maui Nui complex had not appeared (Figure 1). Diversification on the younger islands (Maui and Hawaii) seems to have occurred shortly after each of these islands appeared (Figure S2).

The ecological affinities of the Hawaiian *Ariamnes* are closely linked to their color. While there is considerable variability in both color and form (extension of the abdomen), three distinct ecomorphs can be recognized (Figure 2): gold, dark, and matte white. Each ecomorph is cryptic against the substrate on which the spider is found. Given the exclusively nocturnal behavior of the spiders and their very limited visual capacity, diurnal predation is the most likely selective pressure responsible for the close color matching [27]. Birds, as the only native vertebrates in Hawaiian forests except for one bat, are by far the dominant arthropod predators throughout the native ecosystems of Hawaii, with early observations suggesting that for spiders, "being

Table 1. The Hawaiian *Ariamnes* Stick Spiders and Associated Characteristics

Species	n	Island	Habitat	Body Form	Food[a]	Color	Microhabitat[b]	Ecomorph
A. huinakolu	5	K	wet forest	short	F&K	red/dark	in moss	short moss
A. n sp	2	K	wet forest	long	F&K	dark	dead fern/rock	dark
A. kahili	7	K	wet forest	long	F&K	gold	under leaves	gold
A. uwepa	6	O	wet/mesic	long	F	gold	under leaves	gold
A. n. sp.	2	O	mesic/ dry	long	F	white	in lichen[c]	matte white
A. makue	6	O	mesic	long	F&K	br-blk/dark	dead ferns	dark
A. poele	6	Mo	wet forest	long	F	br-blk/dark	dead ferns	dark
A. n. sp	2	Mo	wet forest	long	F	gold	under leaves	gold
A. melekalikimaka	7	WM	wet forest	medium	F&K	gold	under leaves	gold
A. n. sp	2	WM	wet forest	long	F	br-blk/dark	dead ferns	dark
A. corniger	8	EM	dry and wet	long	F	white	in lichen[c]	matte white
A. alepeleke	6	EM	wet forest	medium	F	gold	under leaves	gold
A. laau	7	EM	wet forest	long	F&K	br-blk/dark	dead ferns	dark
A. waikula	7	H	wet forest	long	F	gold	under leaves	gold
A. hiwa	8	H	wet forest	long	F	br-blk/dark	rock crevices	dark

K, Kauai; O, Oahu; M, Molokai; WM, West Maui; EM, East Maui; H, Hawaii; F, free-living state; K, kleptoparasitic association; br-blk, brown-black. See also Table S1.[a]At night, species were found either in free-living state or in kleptoparasitic association with *Orsonwelles* spider webs. In some situations, spiders have only been collected in inactive state during the day, in which case feeding associations are unknown.
[b]By day, species were found camouflaged against specific microhabitats.
[c]Fruticose lichen, genera *Usnea* (Parmeliaceae) or *Alectoria* (Alectoriaceae).

so favourite a food of passerine birds, the arboreal forms especially, were they not concealed by day, would have had little chance of survival'' [28]. Additional predators include other spiders, though because most of these are also nocturnal [29], they are unlikely to play a major role in dictating diurnal substrate color matching of the Hawaiian *Ariamnes*. Predatory flies in the genera *Lispocephala* and *Discretomyia* (Muscidae) are also known to prey on small subfoliar insects [30], though they are not known to target relatively large spiders, such as *Ariamnes*.

Considering individual ecomorphs of the Hawaiian *Ariamnes*, the shiny gold form is the most common and generally found under leaves, where it is very cryptic. It exhibits a color polymorphism that characterizes many arthropods that are found exposed under leaves [31], with most individuals displaying plain

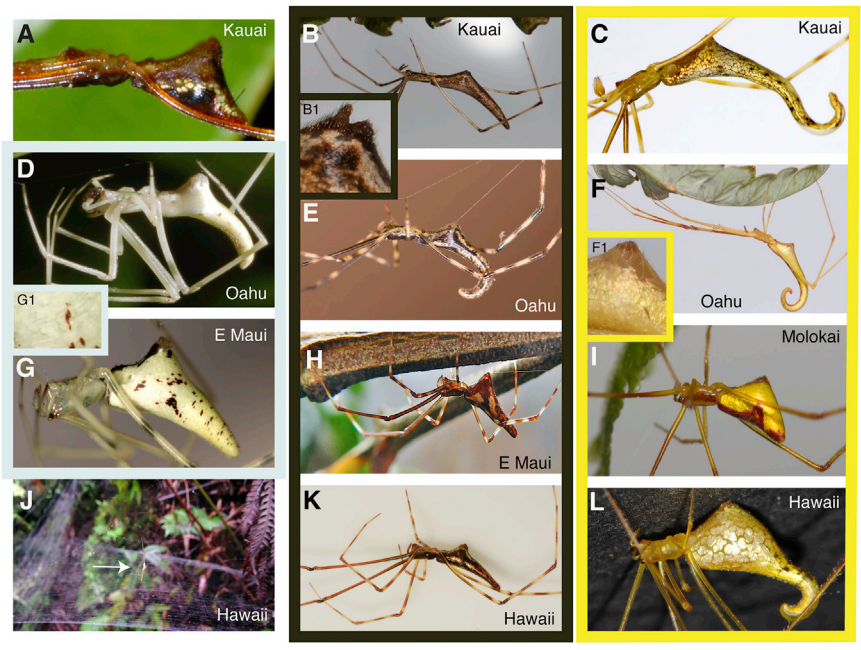

Figure 2. Ecological Forms of the Hawaiian *Ariamnes*

Colored boxes around images show the different ecomorphs: matte white, dark, and gold.
(A) *A. huinakolu*; Kauai, Makalehas; July 2008.
(B) *A. sp.*; Kauai, Pihea; November 2016.
(C) *A. kahili*; Kauai, Wailua River; November 2016.
(D) *A. sp.*; Oahu, Pahole; August 2008.
(E) *A. makue*; Oahu, Kaala; November 2016.
(F) *A. uwepa*; Oahu, Poamoho; November 2016.
(G) *A. corniger*; East Maui; November 2016.
(H) *A. laau*; East Maui; July 2013.
(I) *A. sp.*; Molokai; November 2016.
(J) *A. waikula* on web of *Orsonwelles*; Hawaii; July 2013.
(K) *A. hiwa*; Hawaii; July 2014.
(L) *A. waikula*; Hawaii, Saddle Road; July 2013.
Note that all of the gold forms—(C), (F), (I), and (L)—can exhibit color polymorphism, with red superimposed on the gold, as shown in (I). Photo credits: G. Roderick, (A–J), A. Rominger, (K), D. Cotoras, (L). Insets (B1, F1, and G1) show details of the guanine structure of the respective forms.

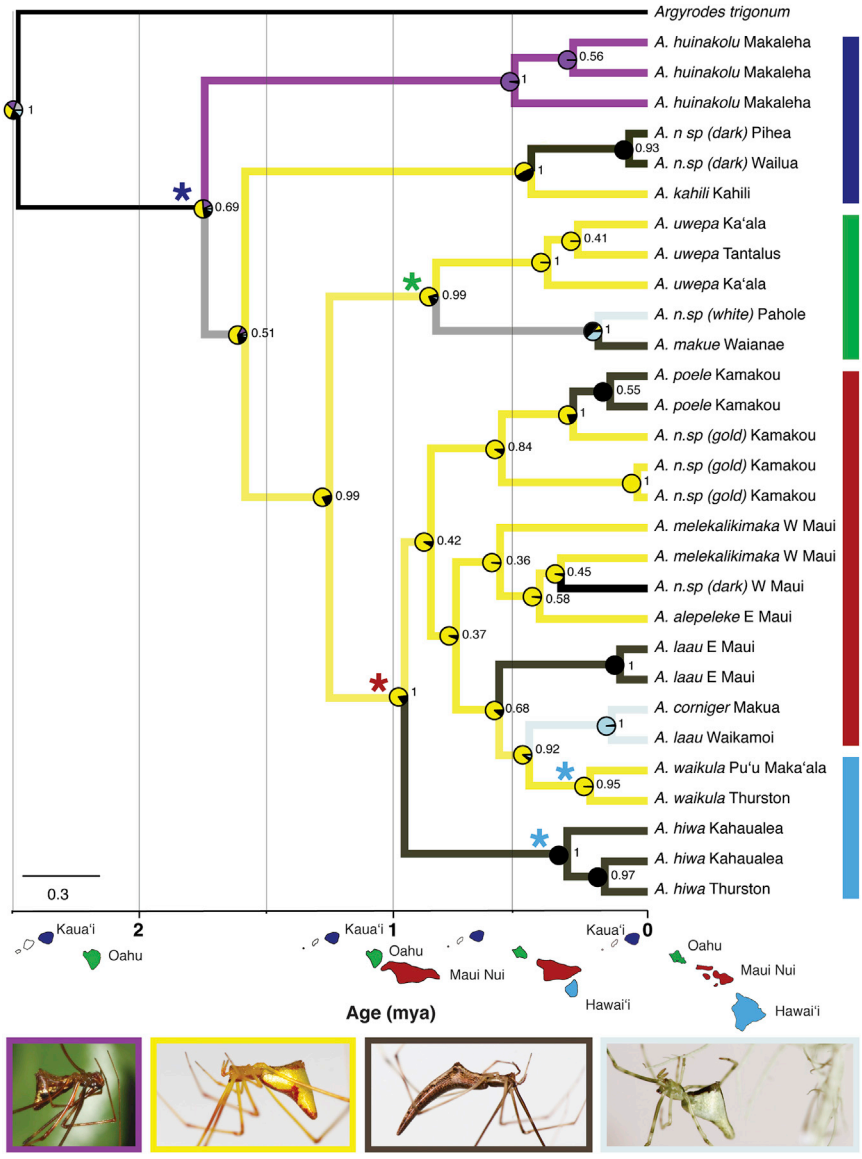

Figure 3. Dated Phylogeny of Hawaiian *Ariamnes* to Show the Time of Divergence Relative to the Age of the Islands

Vertical color bars on the right indicate island: dark blue, Kauai; green, Oahu; red, Maui Nui; light blue, Hawaii. Island arrangements at 2.5, 1.0, 0.5, and 0 mya are shown on the x axis (adapted from [15]). As indicated, both Kauai and Oahu were likely to have been large islands by the time the group reached the islands. Stars indicate colonization of the different islands. Colors of branches indicate ecomorphs (matte white, dark, and gold); purple indicates the unique short-bodied form on Kauai. The likelihood of each ecomorph is indicated at the nodes based on the best-fitting model of equal rates.

See also Figures S2 and S3.

gold but with red frequently found superimposed on the gold (Figure 2I). The dark ecomorph of the Hawaiian *Ariamnes* is generally less common than gold. However, species of the dark ecomorph are almost always found to co-occur with a gold species, though the former invariably occur low in dead ferns or in rocky overhangs, again very cryptic against the dark substrates [13]. The matte white form has so far been found on only two islands, Oahu and Maui, where it may also co-occur with both dark and gold ecomorphs. In contrast to the gold and dark, this form is not shiny. Rather, the color appears matte or dull and provides tight camouflage against filamentous fruticose white lichens (species of *Alectoria* and *Usnea*) that grow on trees and are abundant in the habitats where it occurs [32].

The shiny gold color, as well as the superimposed red marks, can be readily understood in terms of what is known of spider pigmentation, which is frequently the result of dorso-abdominal patterns of yellow, red, and black ommochrome pigments that are responsible for a wide variety of colors, including gold (xanthommatin X), red, violet, and black; the reduced form in spiders is red, and the oxidized form is usually yellow. Ommochromes are deposited in subcuticular hypodermal pigment granule cells that overlay a reflective background of guanine crystals, a metabolic waste product [33, 34], with the combination yielding a gold color.

A key aspect of the color in *Ariamnes*, as with many spiders, lies in the use of guanine to provide a uniformly light background: the cuticle of the abdomen in spiders is thin, which means that for spiders that use pale colors (white, yellow, or green) for camouflage, the darkish color of the spider midgut would show through if guanine were not deposited behind the pale pigments [35]. The guanine covers the gut diverticula, and pigments of yellow, red, and green can be superimposed. The guanine itself can lead to two different kinds of light interaction effects: light scattering and spectral (mirror-like) reflection. Silvery reflection is the result of thin plates that can provide multidirectional reflectance; light scattering is produced by small, cuboid guanine crystals, resulting in a matte-white color [36]. Spiders that live in open habitats tend to make more extensive use of guanine for coloration [35]. For *Ariamnes*, the shiny form of guanine is found where the reflectance appears to be used to accentuate the color of the pigments (yellow and red), or the surroundings, against the underleaf substrate. In contrast, the matte coloration is found when spiders are making use of the white color of guanine itself.

Character reconstructions were used to examine the sequence of evolution of ecomorphs using ACCTRAN [37], which minimizes parallel evolution. The topology indicates that a minimum of four character transformations are required, one with the divergence of the lineage from *A. huinakolu*, the second with *A. makue* from *A. uwepa*, the third within the polytomy of species on Maui/Hawaii that show both gold and dark ecomorphs, and the fourth within

A. corniger, which is matte white and largely confined to dry forests. Character evolution was further examined using likelihood-based approaches as implemented in the R package "ape" [38, 39], with the likelihood of each morphology indicated on the nodes (Figure 3). Additional analyses using the R package "phytools" [40] application of stochastic character mapping yielded similar results (Figure S3). These results show that the radiation of Hawaiian *Ariamnes* is associated with repeated evolution of ecologically equivalent and discrete phenotypes (Figures 2 and 3) in a manner similar to the unrelated spiny-leg *Tetragnatha* [24]. Conversion between forms of the pigments, as described above, together with changes in the structural form of guanine, both appear to have played a role in the evolutionary shifts in the use of shiny and matte colors.

Drivers of Convergence

The current study provides evidence for strong morphological and ecological convergence within the adaptive radiation of Hawaiian *Ariamnes* spiders. Convergence is a common feature of many adaptive radiations, with some lineages showing repeated episodes of adaptation to similar environments. Some of the best-studied examples of this phenomenon include cichlid fish in the African Rift lakes [41], *Anolis* lizards in the Caribbean [42], and *Mandarina* snails of the Bonin Islands [43], where highly deterministic sets of ecomorphs have evolved independently on each lake or island. Likewise, among Hawaiian spiders, adaptive radiation in the genus *Tetragnatha* is characterized by convergence between islands in microhabitat selection [2], as well as in web-building behaviors and prey capture [44, 45].

Despite the prevalence of convergence, demonstrations of parallel evolution of ecomorphs giving rise to almost identical sets of taxa evolving repeatedly in the same area have been found in only rather few lineages of terrestrial organisms, most notably Caribbean *Anolis* lizards [1, 42], Hawaiian long-jawed spiders in the spiny-leg clade of *Tetragnatha* [2], and now also in the Hawaiian *Ariamnes*, as shown here. Therefore, with these three independent lineages, we can ask whether there might be an underlying mechanism that leads to the similar patterns of predictably repeated evolution in the course of adaptive radiation. Importantly, both the *Anolis* lizards and *Tetragnatha* spiders have sister lineages that show adaptive radiation, but without the repeated evolution of ecomorphs; for *Anolis* lizards, repeated formation of the same set of ecomorphs is demonstrated in lineages from the Caribbean islands, but not elsewhere, at least not in such a discrete manner [46]. For *Tetragnatha* spiders, repeated evolution of the same ecomorph is evident in the spiny-leg clade, in which most forest habitats are occupied by a set of three or four distinct ecomorphs, with replicated evolution of almost identical sets on different islands [2]. However, such repeated evolution of ecomorphs is not found in the equally (if not more) diverse sister lineage of web builders [47].

These contrasting patterns between related radiations and similar patterns across independent lineages (*Anolis*, *Ariamnes*, and *Tetragnatha*) prompt the question: what are the common denominators underlying parallel evolution of discrete sets of ecomorphs? Although Caribbean *Anolis* are diurnal while both the Hawaiian *Ariamnes* and *Tetragnatha* spider lineages are nocturnal, all three use daylight microhabitats that are defined by the ecomorph, with selection for camouflage against the limited number of island predators playing a key role [42]. The limited predatory repertoire on islands has been suggested to explain why island *Anolis* show repeated evolution of ecomorphs while mainland lineages do not. In the case of Hawaiian spiders, the equivalent contrast is between the tendency for ecomorph formation in the *Ariamnes* and spiny-leg *Tetragnatha*, which are both free living, but not in the web-building *Tetragnatha* lineage [47]. In the latter, selective pressures are likely to be quite different, given that sites for web placement demand strict habitat requirements, prompting spiders to hide near these locations. By contrast, free-living spiders find themselves exposed in different locations each day. Taken together, several common associations with known occurrences of repeated evolution of ecomorphs emerge: a remote insular habitat, parallel developmental and genetic systems, and a rugged and sparse adaptive landscape—the latter due to selection acting to camouflage animals that do not otherwise hide—against a defined and limited set of predators.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- CONTACT FOR REAGENT AND RESOURCE SHARING
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Taxonomic sampling
- METHOD DETAILS
  - DNA extraction and manipulation
- QUANTIFICATION AND STATISTICAL ANALYSIS
  - Phylogenetic analyses
  - Time of divergence
  - Ecological Shifts
- DATA AND SOFTWARE AVAILABILITY

SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures, one table, and two data files and can be found with this article online at https://doi.org/10.1016/j.cub.2018.01.083.

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AUTHOR CONTRIBUTIONS

Conceptualization: R.G.G., S.P.B., M.S.B., M.A.J.R., and G.K.R.; Methodology: R.G.G., S.P.B., M.S.B., M.A.J.R., and G.K.R.; Formal Analysis: R.G.G., M.S.B., and G.K.R.; Investigation: R.G.G., S.P.B., M.S.B., M.A.J.R., and G.K.R.; Resources: R.G.G., S.P.B., M.S.B., M.A.J.R., and G.K.R.; Data

Curation: R.G.G. and M.S.B.; Writing—Original Draft: R.G.G.; Writing—Review and Editing: R.G.G., S.P.B., M.S.B., M.A.J.R., and G.K.R.; Visualization: R.G.G., M.S.B., and G.K.R.; Supervision: R.G.G.; Project Administration: R.G.G.; Funding Acquisition: R.G.G.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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REFERENCES

1. Mahler, D.L., Ingram, T., Revell, L.J., and Losos, J.B. (2013). Exceptional convergence on the macroevolutionary landscape in island lizard radiations. Science *341*, 292–295.
2. Gillespie, R. (2004). Community assembly through adaptive radiation in Hawaiian spiders. Science *303*, 356–359.
3. Gillespie, R.G., Baldwin, B.G., Waters, J.M., Fraser, C.I., Nikula, R., and Roderick, G.K. (2012). Long-distance dispersal: a framework for hypothesis testing. Trends Ecol. Evol. *27*, 47–56.
4. Miller, S.E., and Eldredge, L.G. (1996). Numbers of Hawaiian Species: Supplement 1. Bishop Museum Occasional Papers *45*, 8–17.
5. Wagner, W.L., Herbst, D.R., and Sohmer, S.H. (1999). Manual of the Flowering Plants of Hawai'i*Volume 1 and 2* (University of Hawai'i and Bishop Museum Press).
6. Clague, D.A. (1996). The growth and subsidence of the Hawaiian-Emperor volcanic chain. In The origin and evolution of Pacific island biotas, New Guinea to eastern Polynesia: patterns and processes, M.P. Poland, T.J. Takahashi, and C.M. Landowski, eds. (Amsterdam: SPB Academic), pp. 35–50.
7. Price, J.P., and Clague, D.A. (2002). How old is the Hawaiian biota? Geology and phylogeny suggest recent divergence. Proc. Biol. Sci. *269*, 2429–2435.
8. Wagner, W.L., and Funk, V.A., eds. (1995). Hawaiian Biogeography: Evolution on a Hot Spot Archipelago (Washington, DC: Smithsonian Institution Press).
9. Shaw, K.L., and Gillespie, R.G. (2016). Comparative phylogeography of oceanic archipelagos: Hotspots for inferences of evolutionary process. Proc. Natl. Acad. Sci. USA *113*, 7986–7993.
10. Rundell, R.J., and Price, T.D. (2009). Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. Trends Ecol. Evol. *24*, 394–399.
11. Hormiga, G., Arnedo, M., and Gillespie, R.G. (2003). Speciation on a conveyor belt: sequential colonization of the hawaiian islands by *Orsonwelles* spiders (Araneae, Linyphiidae). Syst. Biol. *52*, 70–88.
12. Gillespie, R.G., and Baldwin, B.G. (2010). Island Biogeography of Remote Archipelagoes Interplay between Ecological and Evolutionary Processes. In The Theory of Island Biogeography Revisited, pp. 358–387.
13. Gillespie, R.G., and Rivera, M.A. (2007). Free-living spiders of the genus *Argyrodes* (Araneae: Theridiidae) in Hawaii. J. Arachnol. *35*, 11–37.
14. Hormiga, G. (2002). *Orsonwelles*, a new genus of giant linyphiid spiders (Araneae) from the Hawaiian Islands. Invertebr. Syst. *16*.
15. Carson, H.L., and Clague, D.A. (1995). Geology and biogeography of the Hawaiian Islands. In Hawaiian Biogeography Evolution on a Hot Spot Archipelago, W.L. Wagner, and V.A. Funk, eds. (Washington, DC: Smithsonian Institution Press), pp. 14–29.
16. Exline, H., and Levi, H.W. (1962). American spiders of the genus *Argyrodes* (Araneae, Theridiidae). Bull. Mus. Comp. Zool. *127*, 75–204.
17. Agnarsson, I. (2004). Morphological phylogeny of cobweb spiders and their relatives (Araneae, Araneoidea, Theridiidae). Zool. J. Linn. Soc. *141*, 447–626.
18. Whitehouse, M. (2011). Kleptoparasitic spiders of the subfamily Argyrodinae: a special case of behavioural plasticity. In Spider Behaviour: Flexibility and Versatility, M.E. Herberstein, ed. (Cambridge: Cambridge University Press).
19. Whitehouse, M., Agnarsson, I., Miyashita, T., Smith, D., Cangialosi, K., Masumoto, T., Li, D., and Henaut, Y. (2002). *Argyrodes*: phylogeny, sociality and interspecific interactions—a report on the *Argyrodes* symposium, Badplaas 2001. J. Arachnol. *30*, 238–245.
20. Cangialosi, K.R. (1997). Foraging versatility and the influence of host availability in *Argyrodes trigonum* (Araneae, Theridiidae). J. Arachnol. *25*, 182–193.
21. Whitehouse, M.E.A. (1997). The benefits of stealing from a predator: foraging rates, predation risk, and intraspecific aggression in the kleptoparasitic spider *Argyrodes antipodiana*. Behav. Ecol. *8*, 665–667.
22. Su, Y.-C., and Smith, D.R. (2014). Evolution of host use, group-living and foraging behaviours in kleptoparasitic spiders: molecular phylogeny of the Argyrodinae (Araneae: Theridiidae). Invertebr. Syst. *28*, 415–431.
23. Schluter, D. (2000). The ecology of adaptive radiation (Oxford University Press).
24. Gillespie, R.G. (1991). Hawaiian spiders of the genus *Tetragnatha*: I. Spiny leg clade. J. Arachnol. *19*, 174–209.
25. Marshall, D.C., Hill, K.B.R., Moulds, M., Vanderpool, D., Cooley, J.R., Mohagan, A.B., and Simon, C. (2016). Inflation of molecular clock rates and dates: Molecular phylogenetics, biogeography, and diversification of a global Cicada radiation from Australasia (Hemiptera: Cicadidae: Cicadettini). Syst. Biol. *65*, 16–34.
26. Brower, A.V. (1994). Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. Proc. Natl. Acad. Sci. USA *91*, 6491–6495.
27. Oxford, G.S., and Gillespie, R.G. (2001). Portraits of Evolution: Studies of Coloration in Hawaiian Spiders: The discrete color polymorphisms in spiders allow the study of evolution "in action". Bioscience *51*, 521–528.
28. Perkins, R.C.L. (1913). Introduction: Being a Review of the Land-Fauna of Hawaii, *Volume 1* (Cambridge: Cambridge University Press).
29. Gillespie, R.G. (2015). Island time and the interplay between ecology and evolution in species diversification. Evol. Appl. *9*, 53–73.
30. Hardy, D.E. (1981). Diptera: Cyclorrhapha IV, Series Schizophora Section Calyptratae, *Volume 14* (Honolulu, Hawaii: University of Hawai'i Press).
31. Cotoras, D.D., Brewer, M.S., Croucher, P.J., Oxford, G.S., Lindberg, D.R., and Gillespie, R.G. (2016). Convergent evolution in the colour polymorphism of *Selkirkiella* spiders (Theridiidae) from the South American temperate rainforest. Biol. J. Linn. Soc. Lond. *120*, 649–663.
32. Smith, C.W. (1984). Hawaii's Alectorioid Lichens. Pac. Sci. *38*, 249–252.
33. Insausti, T.C., and Casas, J. (2008). The functional morphology of color changing in a spider: development of ommochrome pigment granules. J. Exp. Biol. *211*, 780–789.
34. Oxford, G.S., and Gillespie, R.G. (1998). Evolution and ecology of spider coloration. Annu. Rev. Entomol. *43*, 619–643.
35. Oxford, G. (1998). Guanine as a colorant in spiders: development, genetics, phylogenetics and ecology. In Proceedings of the 17th European Colloquium of Arachnology, Edinburgh, P.A. Selden, ed. pp. 121–131.
36. Levy-Lior, A., Shimoni, E., Schwartz, O., Gavish-Regev, E., Oron, D., Oxford, G., Weiner, S., and Addadi, L. (2010). Guanine-Based Biogenic Photonic-Crystal Arrays in Fish and Spiders. Adv. Funct. Mater. *20*, 320–329.
37. Swofford, D.L., and Maddison, W.P. (1992). Parsimony, character-state reconstructions, and evolutionary inferences. Systematics, historical ecology, and North American freshwater fishes *1*.
38. Paradis, E., Claude, J., and Strimmer, K. (2004). APE: analyses of phylogenetics and evolution in R language. Bioinformatics *20*, 289–290.

39. R Development Core Team (2016). R: A language and environment for statistical computing (Vienna, Austria: R Foundation for Statistical Computing).
40. Revell, L.J. (2012). phytools: an R package for phylogenetic comparative biology (and other things). Methods Ecol. Evol. *3*, 217–223.
41. Brawand, D., Wagner, C.E., Li, Y.I., Malinsky, M., Keller, I., Fan, S., Simakov, O., Ng, A.Y., Lim, Z.W., Bezault, E., et al. (2014). The genomic substrate for adaptive radiation in African cichlid fish. Nature *513*, 375–381.
42. Losos, J.B. (2009). Lizards in an Evolutionary Tree: Ecology and Adaptive Radiaiton of Anoles (Berkeley, CA: University of California Press).
43. Davison, A., and Chiba, S. (2008). Labile ecotypes accompany rapid cladogenesis in an adaptive radiation of *Mandarina* (Bradybaenidae) land snails. Biol. J. Linn. Soc. Lond. *88*, 269–282.
44. Blackledge, T.A., Binford, G.J., and Gillespie, R.G. (2003). Resource use within a community of Hawaiian spiders (Araneae: Tetragnathidae). Ann. Zool. Fenn. *40*, 293–303.
45. Blackledge, T.A., and Gillespie, R.G. (2004). Convergent evolution of behavior in an adaptive radiation of Hawaiian web-building spiders. Proc. Natl. Acad. Sci. USA *101*, 16228–16233.
46. Irschick, D.J., Vitt, L.J., Zani, P.A., and Losos, J.B. (1997). A comparison of evolutionary radiations in mainland and Caribbean Anolis lizards. Ecology *78*, 2191–2203.
47. Gillespie, R.G. (2013). Adaptive radiation: convergence and non-equilibrium. Curr. Biol. *23*, R71–R74.
48. Arnedo, M.A., Coddington, J., Agnarsson, I., and Gillespie, R.G. (2004). From a comb to a tree: phylogenetic relationships of the comb-footed spiders (Araneae, Theridiidae) inferred from nuclear and mitochondrial genes. Mol. Phylogenet. Evol. *31*, 225–245.
49. Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., and Flook, P. (1994). Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. *87*, 651–701.
50. Whiting, M.F., Carpenter, J.C., Wheeler, Q.D., and Wheeler, W.C. (1997). The Strepsiptera problem: phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. Syst. Biol. *46*, 1–68.
51. Folmer, O., Hoeh, W., Black, M., and Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. *3*, 294–299.
52. Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., and Lopez, R. (2007). Clustal W and Clustal X version 2.0. Bioinformatics *23*, 2947–2948.
53. Katoh, K., Misawa, K., Kuma, K., and Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. *30*, 3059–3066.
54. Katoh, K., and Toh, H. (2008). Recent developments in the MAFFT multiple sequence alignment program. Brief. Bioinform. *9*, 286–298.
55. Maddison, W.P., and Maddison, D.R. (2017). Mesquite: a modular system for evolutionary analysis. Version 3.2.
56. Tanabe, A.S. (2011). Kakusan4 Version 4.0.
57. Kück, P., and Meusemann, K. (2010). FASconCAT: Convenient handling of data matrices. Mol. Phylogenet. Evol. *56*, 1115–1118.
58. Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., and Huelsenbeck, J.P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. *61*, 539–542.
59. Stamatakis, A. (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics *22*, 2688–2690.
60. Silvestro, D., and Michalak, I. (2012). raxmlGUI: a graphical front-end for RAxML. Org. Divers. Evol. *12*, 335–337.
61. Drummond, A.J., Ho, S.Y., Phillips, M.J., and Rambaut, A. (2006). Relaxed phylogenetics and dating with confidence. PLoS Biol. *4*, e88.
62. Drummond, A.J., and Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. *7*, 214.
63. Rambaut, A. (2014). FigTree 1.4.2 (Institute of Evolutionary Biology, Univ. Edinburgh).
64. Arnedo, M.A., Agnarsson, I., and Gillespie, R.G. (2007). Molecular Insights into the phylogenetic structure of the spider genus *Theridion* (Araneae, Theridiidae) and the origin of the Hawaiian *Theridion*-like fauna. Zool. Scr. *36*, 337–352.
65. Hedin, M.C., and Maddison, W.P. (2001). Phylogenetic utility and evidence for multiple copies of elongation factor-1α in the spider genus *Habronattus* (Araneae: Salticidae). Mol. Biol. Evol. *18*, 1512–1521.
66. Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). Molecular cloning: A laboratory manual, Second Edition (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press).

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological Samples		
Hawaiian stick spiders in the genus *Ariamnes*	This paper	Essig Museum of Entomology, UC Berkeley
Deposited Data		
Molecular sequences	This paper	GenBank: MG548268–GenBank: MG548308
Phylogenetic data files and resulting trees	This paper	TreeBASE: 22193, TreeBASE: 22194
Oligonucleotides		
A: CGCCTGTTTATCAAAAACAT B2: CTCCGGTTTGAACTCAGATCA	[48]	N/A
C1-J-1718: GGAGGATTTGGAAATTGATTAGTTCC C1-N-2191: CCCGGTAAAATTAAAATATAAACTTC	[49]	N/A
28SA: GACCCGTCTTGAAACACGGA 28SB: TCGGAAGGAACCAGCTACTA	[50]	N/A
LCOI 1498: GGTCAACAAATCATAAAGATATTGG LCOI 2198: TAAACTTCAGGGTGACCAAAAAATCA	[51]	N/A
efF1ArgF: GTTSCATTTGTWCCTATTTCTG efaArgRL: CAGAAACATTCTTAACATKGAA	This paper	N/A
Software and Algorithms		
ClustalX	[52]	N/A
MAFFT 7	[53, 54]	N/A
Mesquite 3	[55]	N/A
KAKUSAN4	[56]	N/A
FASconCAT	[57]	N/A
MRBAYES v.3.2.3	[58]	N/A
RAXML v.7.4.2 via RAXMLGUI	[59, 60]	N/A
BEAST v.1.8.2	[61, 62]	N/A
FigTREE	[63]	N/A

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests of resources should be directed to and will be fulfilled by the Lead Contact, Rosemary G. Gillespie (gillespie@berkeley.edu).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Taxonomic sampling

Hawaiian *Ariamnes* taxa were sampled over the range of all species across the Hawaiian Archipelago (Table 1, with additional locality details in Table S1). Each sampled specimen was retained as a voucher in 95% ethanol to be deposited at the UC Berkeley Essig Museum of Entomology. Hawaiian specimens were collected from islands of Kauai, Oahu, Maui, Molokai and Hawaii (Figure 1). Sexually mature spiders were identified to species using a published key [13]. Specimens representing all 11 of the described Hawaiian species as well as 4 undescribed species were collected and included in subsequent analyses (Table 1). For each taxon, two individuals of each species were included wherever possible. A total of 34 specimens of Hawaiian *Ariamnes* collected from the field were analyzed for DNA sequences (Table S1).

Outgroup selection relied on published studies on the morphological [17] and molecular phylogeny of spiders in the Theridiidae [48, 64] and Argyrodinae [22].

METHOD DETAILS

DNA extraction and manipulation

Genomic DNA was extracted from 1-4 legs of freshly collected specimens fixed in 95% ethanol. Partial fragments of the mitochondrial gene cytochrome *c* oxidase 1 (CO1) and either (i) 16S and 28S ribosomal RNA for assessment of monophyly and placement of the lineage relative to others; and (ii) the nuclear elongation factor (EF1alpha) gene for assessment of relationships within the Hawaiian radiation. COI sequences were amplified and sequenced using the primer pairs: C1-J-1718 and C1-N-2191 [49] (CO1, 472 bp); 16S using the primer pairs *A* and *B2* for the 16S (450 bases, 12864-13417 in *Drosophila*) [48]. The 28S, which was sequenced for 4 representatives of the Hawaiian radiation plus several sequences from GenBank, was amplified using the primer pairs 28SA and 28SB (364 bases, 4066-4393 in *Drosophila*) [50]. Portions of COI were amplified in overlapping fragments using the QIAGEN DNAeasy Tissue kit (QIAGEN, Inc., Valencia, CA) and either universal primers LCOI 1498 and LCOI 2198 (see Key Resources Table) [51], to produce a ~700 base-pair (bp) fragment, or universal primers C1-J-1718 and C1-N-2191 (see Key Resources Table) [49], generating a ~473 bp fragment. PCR conditions to amplify either COI segments included an initial 95°C denaturation of 90 s, followed by 35 cycles of 30 s at 94°C, 40 s ranging from 45°C to 55°C, 45 s at 72°C, followed by a final 10 minute 72°C extension. PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN, Inc., Valencia, CA) and sequenced directly in both directions when possible using either ABI 377 or ABI 310 automatic sequencers (Applied Biosystems, Foster City, CA) with the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit.

Elongation Factor sequences were obtained for all fresh specimens of the Hawaiian lineage, initially using primers tailored for jumping spiders (*Habronattus*; Salticidae) [65] and modifying these to specific primers for the Hawaiian *Ariamnes*: efF1ArgF GTTSCATTTGTWCCTATTTCTG; and efaArgR CAGAAACATTCTTAACATKGAA. PCR amplification used a touch-down protocol with an initial 95°C denaturation for 60 s followed by 16 cycles of 30 s at 95°C, followed by 58°C for 60 s, and 60 s at 72°C, lowering the temperature by 2°C every three cycles. This was followed by 18 cycles of a 95°C denaturation of 30 s followed by 60 s at 42°C, and 60 s at 72°C followed by a final 10 minute extension at 72°C. Double-stranded PCR products were polyacrylamide gel-purified [66] and directly sequenced using ABI dye chemistry on an ABI 377 machine. Both strands were determined for most templates using PCR primers as sequencing primers. TheEF1 primers amplified a single fragment approximately 460 bp in length with a single intron of length 73 bp. Compared to salticids (*Habronattus*), this intron was shorter (140 bp in *Habronattus*), running from 212-287. However, in *A. huinakolu* it was 5 bp shorter (lacking 219-223) and Oahu 2 bp shorter (lacking 233-234). The final concatenated alignment comprised 1,055 nucleotides (464 – EF1; 591 – COI).

Text and chromatogram files produced for each DNA sequence were compiled and edited in Sequencher 3.1 (Gene Codes Corp., Ann Arbor MI). Each text file was compared visually against chromatograms and rechecked against complementary strands. The protein-coding sequences were translated into their corresponding amino acids in order to identify codon positions. These sequences were easily aligned manually due to the fact that COI and Elongation Factor are protein-coding, except for the intron region of EF1, and so could readily be aligned by eye; the intron was also easily alignable as it was the same length in all Hawaiian species for which it was obtained. Regions of 28S, which contained both insertions and deletion, were aligned using the automatic alignment program ClustalX [52] using default options. The aligned matrix was then subject to phylogenetic analyses.

Sequences for each locus were aligned using MAFFT 7 [53, 54] under the default settings and edited in Mesquite 3 [55]. The COI and EF1 alignments were examined for breaks in coding frame by translating to amino acids in Mesquite. Both alignments were trimmed on each end to reduce poorly aligned regions and poor coverage across the dataset.

QUANTIFICATION AND STATISTICAL ANALYSIS

Phylogenetic analyses

We employed Bayesian Inference (BI) analysis to estimate phylogenetic relationships among populations and species. Two datasets were analyzed independently: First, to assess the placement of Hawaiian *Ariamnes* within the Argyrodinae, we used a single species of Hawaiian *Ariamnes*, *A. corniger*, and examined the placement of this species relative to a larger dataset across the subfamily [22] (Figure S1 and Data S2). Second, we examined a smaller Hawaiian *Ariamnes* dataset of COI and EF1a genes.

For the concatenated (COI and EF1a) data, best-fit codon partitioning schemes and nucleotide substitution models were selected according to the Bayesian Information Criterion (BIC4, samples sizes equal to number of sequences), implemented in KAKUSAN4 4.0.2011.05.28 [56]. The datasets were concatenated for downstream analyses using FASconCAT 1.0 [57]. BI analysis was conducted in MRBAYES v.3.2.3 [58], which involved two concurrent runs with four simultaneous chains of $2x10^7$ generations, sampled every 1,000 generations. The first 25% of the posterior distribution of trees was discarded as burn-in. Likelihood values for all post-analysis trees and parameters were evaluated for convergence using the "sump" command in MRBAYES and the program TRACER v. 1.6 (http://tree.bio.ed.ac.uk/software/tracer/). We then used the MRBAYES "sumt" command to generate a majority-rule consensus of the remaining trees. Maximum Likelihood (ML) phylogenies were estimated in RAXML v.7.4.2 [59] via RAXMLGUI [60] comprising 1,000 random addition sequence (RAS) replicates and rapid bootstrapping under the GTR model (partitioned by gene and codon position when appropriate).

To test for compatibility between the two markers, we estimated the Bayes Factor between the marginal likelihoods of the phylogenies generated from COI and EF1a data. Each locus was analyzed in independently, unconstrained, and using the KAKUSAN-derived models. The resulting tree for EF1a yielded a polytomy. Therefore, we then took the COI tree and constrained

it to the EF1a dataset. Stepping stone (SS) sampling was used to estimate marginal likelihoods, both constrained and unconstrained, and comprised $1x10^7$ generations sampled every 100 generations. The difference in the value of the COI constrained to the EF1a SS marginal Likelihood (−891.59) and the unconstrained EF1a SS marginal Likelihood (−890.07) was 1.52. Bayes factors < 5 are not considered significant; therefore the loci are compatible and can be concatenated.

Time of divergence

Because of the inevitable circularity of using island age for calibration, we applied a general arthropod molecular clock estimate of 2.3%/myr [26] (i.e., 0.0115 s/s/myr) to the COI partition with a strict clock [25] for the Hawaiian taxa (COI and EF1 tree). Bayesian inference was used following the methods of Drummond et al. [61] implemented in BEAST v.1.8.2 [62] and visualized with FigTREE [63]. Two independent BEAST analyses were conducted and combined using LogCombiner. The first 10% of each run was discarded as burn-in. Convergence of parameters was accessed using Tracer (http://beast.community/tracer), and the trees were summed using TreeAnnotator.

Ecological Shifts

Habitat affinities were scored for all individuals and all species collected for the initial descriptions, as well as subsequent collections for molecular (Table S1) and further ecological (Table 1) data. Collections were conducted both at night, when the animals are active, allowing assessment of feeding associations, i.e., whether free-living or kleptoparasitic, as well as by day, allowing assessment of specific microhabitat affinity.

Habitat affinities were recorded for between 5 and 10 mature animals per species (Table 1). Based on the associations, taxa were divided into 3 ecomorphs based on their daylight associations with microhabitats: *gold*, which are shiny gold, often with red marks, and invariably found under leaves during the day; *dark*, which can be found during the day by beating dead ferns or inside lava/ rocky crevices near the ground; and *matte white*, which are found on lichen covered tree trunks characteristic in particular of dry or mesic forest habitats. Character reconstructions were used to examine the evolution of ecomorphs (Figure 3) using ACCTRAN [37], which minimizes parallel evolution. We also used likelihood-based approaches as implemented in the R package APE [38] with the command ''ace.'' This was performed under the equal rates (ER, np = 1), symmetrical rates (SYM, n = 11), and all rates different (ARD, np = 20) models, and the resulting likelihood values were compared via a likelihood ratio test. In addition, stochastic character mapping was iterated 1,000 times under the ER model using the PHYTOOLS [40] command ''make.simmap.'' This method allows for more complete histories of character changes along branches instead of only at nodes.

DATA AND SOFTWARE AVAILABILITY

All molecular sequence data are available in available in GenBank (GenBank: MG548268 – GenBank: MG548308). Datasets for phylogenetic analyses and resulting trees are avaialable in TreeBASE, (https://treebase.org) for placement of Hawaiian Argyrodinae (TreeBASE submission 22194) and relationships of Hawaiian *Ariamnes* (TreeBASE submission 22193).