Stable isotopes of Hawaiian spiders reflect substrate properties along a chronosequence

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The Hawaiian Islands offer a unique opportunity to test how changes in the properties of an isolated ecosystem are propagated through the organisms that occur within that ecosystem. The age-structured arrangement of volcanic-derived substrates follows a regular progression over space and, by inference, time. We test how well documented successional changes in soil chemistry and associated vegetation are reflected in organisms at higher trophic levels – specifically, predatory arthropods (spiders) – across a range of functional groups. We focus on three separate spider lineages: one that builds capture webs, one that hunts actively, and one that specializes on eating other spiders. We analyze spiders from three sites across the Hawaiian chronosequence with substrate ages ranging from 200 to 20,000 years. To measure the extent to which chemical signatures of terrestrial substrates are propagated through higher trophic levels, we use standard stable isotope analyses of nitrogen and carbon, with plant leaves included as a baseline. The target taxa show the expected shift in isotope ratios of $\delta^{15}N$ with trophic level, from plants to cursorial spiders to web-builders to spider eaters. Remarkably, organisms at all trophic levels also precisely reflect the successional changes in the soil stoichiometry of the island chronosequence, demonstrating how the biogeochemistry of the entire food web is determined by ecosystem succession of the substrates on which the organisms have evolved.

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22 Abstract

23 The Hawaiian Islands offer a unique opportunity to test how changes in the properties of an

24 isolated ecosystem are propagated through the organisms that occur within that ecosystem.

25 The age-structured arrangement of volcanic-derived substrates follows a regular progression

26 over space and, by inference, time. We test how well documented successional changes in soil

27 chemistry and associated vegetation are reflected in organisms at higher trophic levels –

28 specifically, predatory arthropods (spiders) – across a range of functional groups. We focus on

29 three separate spider lineages: one that builds capture webs, one that hunts actively, and one

30 that specializes on eating other spiders. We analyze spiders from three sites across the

31 Hawaiian chronosequence with substrate ages ranging from 200 to 20,000 years. To measure

32 the extent to which chemical signatures of terrestrial substrates are propagated through higher

33 trophic levels, we use standard stable isotope analyses of nitrogen and carbon, with plant

34 leaves included as a baseline. The target taxa show the expected shift in isotope ratios of δ^{15}N

35 with trophic level, from plants to cursorial spiders to web-builders to spider eaters. Remarkably,

36 organisms at all trophic levels also precisely reflect the successional changes in the soil

37 stoichiometry of the island chronosequence, demonstrating how the biogeochemistry of the

38 entire food web is determined by ecosystem succession of the substrates on which the

39 organisms have evolved.

40 Introduction

41 Evolutionary processes are determined in large part by the ecosystems within which they take

42 place. While connecting the processes of evolutionary biology and ecology remains a critical

43 frontier in biological sciences (Matthews et al. 2011), there are few studies that demonstrate

44   how mechanisms driving processes of evolutionary biology and ecosystem science are linked.

45   The current study seeks to understand how organismal diversity may reflect successional shifts

46   in soil chemistry by testing the extent to which organisms at different trophic levels reflect the

47   properties of the substrates on which they occur.

48

49   The Hawaiian Archipelago presents a highly suitable system for studying the link between

50   evolutionary processes and ecosystem properties. The current high islands of Hawaii are

51   arranged sequentially from oldest to youngest, with Kauai, at 5.1 million years, in the far

52   northwest, and Hawai'i Island, at <1 million years, in the southeast (Carson and Clague 1995).

53   This sequential order is a consequence of the archipelago being located on a volcanic hot spot,

54   where magma upwelling from the earth's mantle has formed into large shield volcanoes. At the

55   same time, the tectonic plate on which the islands are situated is moving toward the northwest

56   such that each newly emerging island has appeared to the southeast of its next-oldest neighbor.

57   The resultant, nearly-linear age gradient makes Hawaii an ideal *chronosequence:* a temporally

58   varied system in which the ecosystems of the younger sites are currently developing in a

59   manner assumed to reflect the developmental history of the older sites (Walker et al. 2010).

60   Given fairly precise information on the age of formation and subsequent history across a

61   chronosequence, these systems can provide unprecedented insights into ecosystem

62   development. Thus, chronosequences have added significantly to our understanding of how

63   nutrients change over time (Vitousek 2004) and the impact of changes in soil nutrient availability

64   on plants (Wardle et al. 2008), decomposers (Williamson et al. 2005, Doblas-Miranda et al.

65   2008), above-ground and below-ground ecosystem processes (Wardle et al. 2004), and entire

66   arthropod communities (Gruner 2007).

67

68   Hawaii has served as a chronosequence for detailed studies on the ways in which ecosystem

69   properties and functions change over extended time (Vitousek 2004). Nutrient flow and its

70   impacts on primary producers (trees) have been well characterized in this system. Studies have

71   examined the evolution of soils on substrates of different surficial age (300 y – 4.1 Mya),

72   controlled for elevation, climate, land use history, and canopy vegetation (*Metrosideros*

73   *polymorpha*), with all minerals derived from volcanic ash. An important finding of this work was

74   that soil nitrogen, foliar nutrient availability and productivity start off very low, increase rapidly

75   with substrate age, peak on substrates of intermediate age (*ca.* 20,000 y) on the youngest

76   island, and then decline rapidly on older islands before all but disappearing on the oldest

77   (Vitousek et al. 1989, 1995, 1997). A more recent study found that tree height and canopy

78   nitrogen also peak on intermediate-aged (20,000 y) substrates on the youngest island (Vitousek

79   et al. 2009). Nitrogen isotopes follow a similar pattern, with foliar $\delta^{15}N$ very low at the youngest

80   sites, increasing with substrate age, and highest at a 67,000 y site (Vitousek et al. 1989).

81

82   Geologic history and nutrient flow evidently have important effects on the lowest trophic level –

83   plants – but little is known about how these effects might be propagated through higher trophic

84   levels, i.e. higher-level consumers. At the same time, work on the effects of substrate age on

85   above-ground systems, including whole communities, has shown that community traits such as

86   population species diversity (Gillespie and Baldwin 2010, Lim and Marshall 2017), genetic

87   structure (Roderick et al. 2012), and network modularity (Rominger et al. 2016) change in a

88   predictable manner across the Hawaiian chronosequence. However, although these

89   community-level studies have markedly enhanced our understanding of the changes in

90   community ecology over time, there has as yet been no attempt to link chemical changes during

91   the evolution and development of soils (and associated ecosystem properties) with the

92   abundance, diversity, and evolutionary histories of above-ground organisms. The current study

93   begins to address this question by testing the effects of substrate age on the biochemistry –

94   isotopic signatures – of secondary consumers (predators) across a Hawaiian chronosequence.

95

96   The application of stable isotope information has revolutionized studies of nutrient flow and

97   niche ecology in a wide range of organisms (e.g. Fry 1988, Hobson and Welch 1992, Muschick

98   et al. 2012). In particular, nitrogen and carbon stable isotopes have been found to reflect trophic

99   position: both δ^{15}N and δ^{13}C tend to increase in a predictable manner with each successive

100 trophic level (Post 2002, but see deVries et al. 2015). Stable isotopes have also been used to

101 track nutrient flow, climatic shifts, and migration patterns in a variety of ecological systems (Best

102 and Schell 1996, Chamberlain et al. 1997, Iacumin et al. 2005, McMahon et al. 2016). The

103 current study uses stable isotopes of N and C to assess the extent to which entire food webs

104 are influenced by the chemistry of their habitats. We chose to focus on spiders because they

105 are mobile generalist predators and encompass multiple trophic levels, from feeding on

106 herbivorous insects to specializing on the highest predator levels among Hawaiian arthropods

107 (other spiders). This substantial variation in trophic ecology allows us to test how functional and

108 trophic differences are reflected in isotopic signatures, and the extent to which the

109 biogeochemistry of a food web is determined by the chemistry of the immediate substrate. The

110 use of N and C stable isotopes is especially well suited to this study because of the predictable

111 manner in which both elements can reflect trophic position, and because of the importance of

112 nitrogen in ecosystem development (Boring et al. 1988).

113

114 We analyzed Hawaiian spiders belonging to two lineages within the adaptive radiation of long-

115 jawed orb-weavers (*Tetragnatha*, Tetragnathidae) and one lineage within the stick spiders

116 (*Ariamnes*, Theridiidae)*.* The *Tetragnatha* radiation includes *ca.* 60 species, which display a

117 spectacular array of colors, shapes, sizes, behaviors, and ecological affinities not observed

118 elsewhere in the range of this genus (Blackledge and Gillespie 2004, Gillespie 2004, 2015). The

119 radiation consists of two major clades: one that spins webs for prey capture ("web builders"),

120 and another that has lost the web-spinning behavior and instead hunts actively (the "Spiny Leg"

121 clade; Gillespie 1991, 2002). Observational data indicate that both web-building and Spiny Leg

122   *Tetragnatha* feed on a mixture of insect herbivores and predators (Binford 2001), although the

123   exact composition of these spiders' diets has not yet been fully characterized. The Hawaiian

124   *Ariamnes,* currently represented by 11 known species across the Hawaiian Islands (Gillespie

125   and Rivera 2007), are also ecologically diverse and largely araneophagic (i.e., preying on other

126   spiders) (Gillespie et al 2018). Like *Tetragnatha*, these spiders are exclusively nocturnal, and

127   like the Spiny Leg *Tetragnatha*, they hunt without the use of a web.

128

129   Given the contrasting hunting strategies (web-building versus active hunting) and trophic

130   positions (generalist versus araneophagic) across these spider lineages, the three groups vary

131   predictably in their position in the food web, from largely feeding on primary consumers (i.e.,

132   insect herbivores) to exclusively feeding on secondary and higher consumers (i.e., spiders).

133   Within this system, we tested the hypothesis that isotopic signatures of spiders should reflect

134   the biogeochemistry of their respective habitats, from young to older in the Hawaiian

135   chronosequence. Thus, not only should the different spider lineages illustrate now-standard

136   expectations for isotope signatures associated with rising trophic levels, but the trophic ecology

137   of the entire food web should reflect changes in soil chemistry across the chronosequence. In

138   particular, given that δ^{15}N in the soil increases during the building phase of the Hawaiian

139   ecosystems (Vitousek et al. 1997), we expect δ^{15}N to be lowest in the spiders at the youngest

140   (200-750 y) site and highest in the spiders at the oldest (20,000 y) site.

Methods

Study sites

Hawaii's sequential age structure has made it an ideal system for previous studies on soil evolution and nutrient cycling, wherein the Long Substrate Age Gradient (LSAG) was established (Crews et al. 1995, Vitousek 2004). This study focuses on Hawai'i Island, the youngest in the archipelago, because the largest possible range of N availabilities is expected to be found there: soil nitrogen is lowest in the youngest substrates and peaks in the older substrates on Hawai'i Island before declining on the older islands (Vitousek et al. 1995).

Specimens were collected on the windward side of Hawai'i Island under permits from the State of Hawaii Department of Land and Natural Resources (endorsement # FHM14-349) and the National Park Service (study # HAVO-00425). Three sites of different substrate age, chosen for their comparable elevations and climates as well as the overlap of two of the sites ('Ola'a and Laupāhoehoe) with those characterized based on soils (Vitousek et al. 1997), were sampled (see Figure 1). Substrate ages were determined based on data from the United States Geological Survey (Sherrod et al. 2007) and from the LSAG (Crews et al. 1995, Vitousek 2004). All three sites are wet/mesic forest dominated by *Metrosideros polymorpha* and *Acacia koa* in the canopy, with *Cibotium* spp. dominating the understory. The sites range from 1180 to 1390 m in elevation, with mean annual temperatures of 13.9 to 15.4 degrees Celsius and mean annual rainfall of 3035 to 3090 mm (Giambelluca et al. 2014).

Upper Waiakea is a very young site on a 200- to 750-year-old lava flow in a stratified matrix of differently-aged substrates within the Upper Waiakea Forest Reserve, off of Stainback Highway

164   on Mauna Loa. 'Ola'a Forest is on an older lava flow on Kilauea, situated within Hawai'i

165   Volcanoes National Park. The trees in 'Ola'a are rooted in a thick layer of tephra of

166   approximately 2,100 years old (Vitousek 2004), beneath which is an older flow of 5,000-11,000

167   y (see Figure 1). Although the United States Geological Survey (USGS) classifies this substrate

168   as 5,000-11,000 y, we consider its biota to be influenced by the chemical properties of the

169   2,100-year-old tephra in which the forest is rooted. 'Ola'a is therefore approximately one order

170   of magnitude older than Upper Waiakea, yet is located just 11.5 km S of the younger site. This

171   proximity makes the two sites especially useful for measuring effects of habitat age on a small

172   geographical scale. The oldest site in this study is Laupāhoehoe, located in the Laupāhoehoe

173   Experimental Forest Unit on Mauna Kea. While USGS data (Sherrod et al. 2007) estimate the

174   lava flow age at 5,000-11,000 y, the sampling locality overlaps with an LSAG site which has

175   been studied in great detail and whose forest is rooted in a layer of soil believed to be

176   approximately 20,000 y old (Vitousek 2004, Vitousek et al. 2009). We therefore follow the LSAG

177   classification of 20,000 y for Laupāhoehoe.

178

179   Collections at each site were centered at the following coordinates, with searches extending up

180   to 100 m in any direction:

181   •   Upper Waiakea: 19.562° N, 155.272° W

182   •   'Ola'a: 19.462° N, 155.248° W

183   •   Laupāhoehoe: 19.922° N, 155.301° W

184

Specimen collection

185

186   Six species of *Tetragnatha* spiders (*Tetragnatha anuenue*, *T. brevignatha*, *T. hawaiensis*, *T.*

187   *perkinsi*, *T. quasimodo* and the undescribed species *T.* "golden dome") and two species of

188   *Ariamnes* spiders (*Ariamnes hiwa* and *A. waikula*) were collected in the field from 11 March to

189   18 April 2015, and from 6 to 15 February 2016. All study species are morphologically distinct

190   and can be easily identified in the field. Plant samples were leaves of dominant or common

191   forest vegetation (*Metrosideros polymorpha*, *Cibotium* spp. and *Cheirodendron* spp.), all of

192   which are also easily identified on sight. After a preliminary analysis, it was determined that leaf

193   litter should be added to the study in order to help explain differences in carbon isotope values.

194   Unfortunately, permitting and time constraints only allowed for leaf litter to be collected from the

195   youngest site (Upper Waiakea) on 15 October 2016.

196

197   Spiders were individually hand-captured into clean plastic snap-cap vials which were labeled on

198   the outside with unique identifiers, while plant leaves were clipped off with scissors and stored in

199   labeled paper envelopes. In Upper Waiakea, leaf litter was also collected from the bases of *M.*

200   *polymorpha* trees and placed in paper envelopes. Leaves were air-dried in their envelopes in a

201   sealed container of silica for three weeks prior to processing. Leaf litter was dried in a 60° C

202   oven overnight.

203

204   Spiders were photographed up close using a Nikon D5200 with an AF-S DX Micro-NIKKOR 40

205   mm f/2.8 g lens and a Speedlight SB-400 flash from both dorsal and lateral angles. This created

206   a photographic voucher and allowed for visual identification of species, sex, and maturity.

207   Spiders were killed overnight in a freezer and air-dried in separate snap cap vials, each with one

208   clean bead of silica gel, before being transferred to individual 2-mL centrifuge tubes.

209

210   Stable isotope analysis

211   Individual dried spiders were weighed into 9 x 5 mm pressed tin capsules for isotopic analysis.

212   To optimize N content for isotopic analysis, a 1.5-mg mass was recommended for each sample

213   (S. Mambelli, UC Berkeley Center for Stable Isotope Biogeochemistry, pers. comm., 2014). Due

214   to the spiders' small body size (ranging from ca. 0.4 to 5 mg dry weight for the majority of

215   specimens), it was not feasible to obtain sufficient material from individual body parts, although

216   it has been found that different spider tissues can undergo different isotopic turnover rates

217   (Belivanov and Hambäck 2015). Therefore, in order to control for possible variation in isotopic

218   turnover among tissue types, the spiders' entire bodies were analyzed. When spiders exceeded

219   2.5 mg dry weight, they were homogenized (powdered and mixed) with a mortar and pestle, and

220   a 1.5-mg sample of the homogenized tissue was used. For smaller spiders (< 2.5 mg), the

221   whole intact body was packed into the tin capsule in order to avoid excessive loss of material.

222   Plant leaves were individually homogenized in a Mini-Beadbeater (BioSpec Model 8) in 7-mL

223   tubes with stainless steel ball bearings, then weighed into 9 x 5 mm tin capsules. Due to the

224   relatively low N:C ratio of plants, 6 mg of material was weighed out for each leaf sample. Leaf

225   litter was processed in the same manner as plant leaves.

226

227   Samples were analyzed for nitrogen and carbon content (% dry weight) and nitrogen and

228   carbon stable isotope ratios via elemental analyzer/continuous flow isotope ratio mass

229   spectrometry using a CHNOS Elemental Analyzer (model: Vario ISOTOPE cube, Elementar,

230   Hanau, Germany) coupled with an IsoPrime 100 mass spectrometer (Isoprime Ltd, Cheadle,

231   UK). The isotope ratio is expressed in "delta" notation (in parts per thousand, or ‰ units). The

232   isotopic composition of a material relative to that of a standard on a per mill deviation basis is

233   given by $\delta^{15}N$ (or $\delta^{13}C$) = $(R_{sample}/R_{standard}-1)$ x 1,000, where R is the molecular ratio of heavy to

234   light isotopes. The standard for nitrogen is air. The standard for carbon is V-PDB. The reference

235   material NIST SMR 1547 (peach leaves) was used as calibration standard (long-term precision

236   [since 2000] using this standard is +/- 0.07‰ for both N and C isotope analyses). All isotope

237   analyses were conducted at the Center for Stable Isotope Biogeochemistry at the University of

238   California, Berkeley. Long-term external precision based on reference material NIST SMR

239   1577b (bovine liver) is 0.15‰ and 0.10‰, respectively, for N and C isotope analyses.

240

Data analysis

242   Results from the isotopic analysis were categorized into the following functional groups: "plant"

243   (foliar samples of the genera *Metrosideros*, *Cibotium* and *Cheirodendron*); "Spiny Leg" (Spiny

244   Leg *Tetragnatha* species: *T. anuenue*, *T. brevignatha* and *T. quasimodo*); "web" (*Tetragnatha*

245   *hawaiensis*, *T. perkinsi*, and the undescribed species nicknamed *T.* "golden dome"); and

246   "*Ariamnes*" (*Ariamnes hiwa* and *A. waikula*, the two species found on Hawai'i Island (Gillespie

247   and Rivera 2007)). Although all spider species were initially analyzed separately, the results

248   showed that the data grouped taxa together, largely in accordance with functional group.

249   Because members of one group (e.g. web-builders) were closer to one another than to other

250   groups (e.g. Spiny Leg), we chose to focus on these broader ecological categories – functional

251   groups – rather than species.

252

253   Effects of site and functional group on δ^{15}N and δ^{13}C were tested using a 2-way Anova allowing

254   for interaction (with site and functional group as factors) on R statistical software (version 3.2.2,

255   64-bit). Main effects were then analyzed separately: we tested 1) effect of site within each

256   functional group and 2) effect of functional group within each site. When significant differences

257   were found, pairwise comparisons were made using Tukey's honest significant difference test

258   (Tukey 1949).

259   Results

260   We found a significant interaction between site and functional group for δ^{15}N (F = 8.615, p <

261   0.001), but not for δ^{13}C (F = 1.841, p = 0.092; Table 1). Significant main effects were found for

262   all variables tested: site for δ^{15}N (F = 692.1, p < 0.001), functional group for δ^{15}N (F = 113.6, p <

263   0.001), site for δ^{13}C (F = 55.51, p < 0.001), and functional group for δ^{13}C (F = 95.15, p < 0.001).

264

265   Main effects: Site

266   For δ^{15}N, a significant site effect was found within all functional groups (plants: F = 78.74, p <

267   0.001; Spiny Leg: F = 446.9, p < 0.001; web-builders: F = 216.6, p < 0.001; *Ariamnes*: F =

268   80.87, p < 0.001; Table 2 and Figure 2). We performed a Tukey's HSD test to reveal pairwise

269   differences among sites. δ^{15}N showed a clear pattern of stepwise increase with substrate age

270   (lowest in Upper Waiakea [200-750 y], intermediate in 'Ola'a [2,100 y], and highest in

271   Laupāhoehoe [20,000 y]). This pattern held true for every functional group; the only comparison

272   not found to be significant was 'Ola'a vs. Laupāhoehoe within *Ariamnes* (Tukey's adjusted p =

273   0.203).

274

275   For δ^{13}C, a significant site effect was found within every functional group except for plants

276   (plants: F = 0.7997, p = 0.482; Spiny Leg: F = 5.681, p = 0.005; web-builders: F = 31.91, p <

277   0.001; *Ariamnes*: F = 36.62, p < 0.001; Table 2 and Figure 3). Among the three groups of

278   spiders, δ^{13}C was significantly lower in Laupāhoehoe (20,000 y) than in 'Ola'a (2,100 y).

279   Main effects: Functional group

280   A significant functional group effect was found in all sites for $\delta^{15}N$ (Upper Waiakea: F = 68.38, p

281   < 0.001; 'Ola'a: F = 34.23, p < 0.001; Laupāhoehoe: F = 28.90, p < 0.001; Table 3 and Figure

282   2). A Tukey's HSD test found significant differences among every pair of groups except for the

283   following pairs in Upper Waiakea: web-builders vs. *Ariamnes* (Tukey's adjusted p-value =

284   0.071), web-builders vs. leaf litter (Tukey's adjusted p-value = 0.998), and *Ariamnes* vs. leaf

285   litter (Tukey's adjusted p-value = 0.512).

286

287   For $\delta^{13}C$, a significant functional group effect was found at all three sites (Upper Waiakea: F =

288   36.42, p < 0.001; 'Ola'a: F = 41.29, p < 0.001; Laupāhoehoe: F = 41.48, p < 0.001; Table 3 and

289   Figure 3). At all sites, plants were significantly lower in $\delta^{13}C$ than the next-lowest trophic level,

290   Spiny Leg spiders (consumers); therefore, the results of pairwise comparisons between plants

291   and more distant functional groups (web-builders and *Ariamnes*) are not included in the table of

292   Tukey's HSD results. In 'Ola'a (2,100 y), Spiny Leg spiders had significantly lower $\delta^{13}C$ than

293   either web-builders or *Ariamnes* (Tukey's adjusted p-value < 0.001). In Upper Waiakea (200-

294   750 y), although there was no significant difference between *Ariamnes* and either of the

295   *Tetragnatha* functional groups, the Spiny Leg *Tetragnatha* were significantly lower in $\delta^{13}C$ than

296   web-builders (Tukey's adjusted p-value < 0.001). In Laupāhoehoe (20,000 y), no significant

297   difference was found between any pair of spider groups.

298   # Discussion

299   The results from this study provide a novel perspective on how changes in the substrate

300   chemistry of the terrestrial land surfaces across a chronosequence of developing ecosystems

301   are propagated up through an entire food web. To our knowledge this is also the first study to

302   characterize the isotopic signatures of different ecological groups represented by exemplary

303   adaptive radiations of spiders in Hawaii.

304

305   Parallel shifts in isotopic signature across the chronosequence of
306   ecosystem development

307   Previous work on the ecological characteristics of forests across the chronosequence has

308   detailed the evolution of Hawaiian ecosystems in the context of soil properties and vegetation

309   (Vitousek et al. 1989, 1995, 1997). Our results show that the chemical signatures of nutrient

310   availability that characterize a given site are borne all the way up to the highest trophic levels –

311   top predators – on a Hawaiian chronosequence. Indeed, the δ^{15}N values of spiders perfectly

312   match expectations for their respective habitats. Where nitrogen is most limited – at the

313   youngest site (Upper Waiakea) – spiders have the lowest values of δ^{15}N; as substrate age and

314   nitrogen availability increase, so too does the δ^{15}N of spiders. This makes sense physiologically

315   because when nitrogen is very limited, the lighter isotope (^{14}N) is not as easily lost in reactions,

316   and is instead retained at a greater rate in an organism's tissues (Austin and Vitousek 1998).

317   Conversely, when biologically available nitrogen is very abundant, ^{14}N is readily lost (e.g. in

318   excretion), leaving behind a greater proportion of the heavier ^{15}N in the organism's tissues.

319   Thus, our results support the hypothesis that the isotopic signatures of the spiders – as well as

320   the plants – track the changes in the geological age of the islands (Sherrod et al. 2007) and the

321   associated changes in nitrogen in soils across the Hawaiian chronosequence measured by

322   Vitousek et al. (1997).

323

324   While it might not be surprising that the increase in δ^{15}N in the spiders tracks the increases in

325   plants across the geological gradient, the fact that the relationship is so tight is remarkable, as it

326   suggests that even higher-level consumers (predators) reflect the δ^{15}N of the immediate site.

327   This result is especially notable because it was found in cursorial animals, which, because of

328   their mobility, might be expected to show only a weak association with the substrates on which

329   they were collected. Instead, the spiders carry clear signatures of their immediate ecosystem.

330   Given that the sites that were sampled are in very close proximity (11.5 km between 'Ola'a and

331   Upper Waiakea), and not separated by any significant physical barrier, the results suggest an

332   extraordinary level of isolation among spider populations. This has implications for the

333   mechanisms by which the *Tetragnatha* adaptive radiation may have arisen: Isolation between

334   populations separated by short distances can serve as a crucible for evolution (Carson et al.

335   1990). Perhaps the same mechanisms that are currently at work on Hawaii's youngest Island

336   also led to the rise of the approximately 60 endemic *Tetragnatha* species found across the

337   archipelago today. Results for *Ariamnes* were similar, all higher than the other spider lineages,

338   and values increasing with substrate age (though the increase from 'Ola'a to Laupahoehoe was

339   not significant, presumably due to a relatively small sample size (n = 9) at these sites).

340   *Ariamnes,* like the *Tetragnatha*, has undergone a substantial, though smaller at 11-16 species,

341   adaptive radiation across the islands (Gillespie et al. 2018).

342

343   The carbon isotope data show a less clear pattern than nitrogen, but nevertheless indicate site-

344   specific differences among the spiders. Notably, the plant samples did not differ significantly in

345     $\delta^{13}C$ among the three sites, suggesting that perhaps $\delta^{13}C$ does not accurately reflect nutrient

346   differences among the substrates. By contrast, $\delta^{15}N$ appears to strongly reflect nutrient

347   availability at the different sites. However, spiders did consistently show higher $\delta^{13}C$ in 'Ola'a

348   (2,100 y) than in Laupāhoehoe (20,000 y). Thus, the relationship between baseline (plant)

349   signatures and higher predator (spider) signatures is weaker in carbon than in nitrogen. This

350   suggests that spiders at the three sites may not be consuming exactly the same assemblages of

351   prey, perhaps due to variations in the availability of different insect (potential prey) species at

352   different sites. A detailed study of the precise compositions of these spiders' diets, using either

353   molecular gut content analysis (e.g. Krehenwinkel et al. 2017) or an isotopic mixing model with

354   robust sampling of the entire arthropod community, could greatly enhance our understanding of

355   the processes that account for the differences in δ^{13}C among spider populations.

356

357   Trophic positions

358   Our stable nitrogen isotope data reflect the different functional roles and trophic positions of the

359   Hawaiian spiders. Our results are consistent with the enrichment of the heavier isotope, ^{15}N, at

360   higher trophic levels, with plants having the lowest values of δ^{15}N, *Tetragnatha* having

361   intermediate values, and the spider-eating *Ariamnes* having the highest. Additionally, we found

362   that the δ^{15}N of the Spiny Leg (cursorial) spiders was consistently lower than that of web-

363   building *Tetragnatha*. A simple explanation for the difference in δ^{15}N in cursorial vs. web-building

364   spiders may be that the different functional groups consume different prey. Cursorial spiders are

365   likely to interact with abundant insect herbivores, while web-builders may trap a larger

366   proportion of flying insects at higher trophic levels, such as hymenopteran or dipteran

367   parasitoids, decomposers, or predators. This dietary difference has been used to explain the

368   phenomenon of a higher δ^{15}N in web-builders compared to cursorial spiders in a forest hedge

369   community (Sanders et al. 2015). Another possible explanation is that the difference is due to

370   the manufacturing of the orb web itself. Given that webs are an excretory product, and that

371   excretion tends to favor the lighter ^{14}N, it may be that the higher levels of "excretion" lead to an

372   enrichment in ^{15}N in the bodies of web-builders compared with cursorial spiders. A number of

373   previous studies suggest such an effect. For example, across a community of web-building

374   riparian spiders, lower δ^{15}N was found in *Miagrammopes* (Uloboridae; Kelly et al. 2015), a

375   genus characterized by a reduced capture web (often just a single line; Lubin et al. 1978), than

376   in other spiders. Likewise, a study of niche width across a guild of spiders showed that cursorial

377   spiders consistently had the lowest $\delta^{15}N$, while orb web spiders had the highest (Sanders et al.

378   2015). In each of these systems, the cursorial spiders showed the highest levels of intraguild

379   predation (i.e. feeding on other spiders), indicating that trophic position itself is insufficient to

380   explain the lower trophic position of the cursorial spiders relative to the web spinners. This

381   observation raises the possibility again that the web spinning process itself leads to $\delta^{15}N$

382   enrichment. However, further data are clearly needed to determine which of these explanations

383   best accounts for the now recurring pattern of higher ^{15}N in web-builders compared with

384   cursorial taxa.

385

386   The patterns we found in $\delta^{13}C$ were less dramatic than in $\delta^{15}N$, but matched expectations. Foliar

387   samples consistently had the lowest $\delta^{13}C$ of all functional groups. Spider values were

388   substantially offset from leaf values – approximately 4-5 per mill higher at all sites – which

389   suggests a complex food chain consisting of many trophic levels below the spiders. This is

390   plausible given that spider are obligate predators (secondary consumers), and therefore must

391   be trophically removed from plants by at least two levels. The conventional wisdom with $\delta^{13}C$ is

392   "you are what you eat" (Hobson et al. 2010), meaning that most organisms are only slightly

393   enriched in ^{13}C relative to their diets, with standard published offsets of less than 1 per mill for

394   each successive trophic level (Post 2002). Indeed, meta-analysis of isotopic studies has found

395   an average discrimination factor of ~ 0.3 per mill from one trophic level to the next within

396   invertebrates (Caut et al. 2009), although it should be noted that many of the invertebrates

397   included in that meta-analysis are aquatic, and no "standard" $\delta^{13}C$ offset for spiders or other

398   terrestrial arthropods has yet been established. In an effort to fill the large gap between plants

399   and spiders, we added samples of leaf litter from Upper Waiakea. The $\delta^{13}C$ of leaf litter fit neatly

400   between leaves and spiders. This is to be expected given that the lighter ^{12}C is lost as respired

401    $^{12}CO_2$ that is produced at a greater rate during decomposition, leaving the remaining litter

402   relatively ^{13}C enriched (Dawson et al. 2002). Furthermore, this finding fits with previous studies

403   of *Tetragnatha* trophic ecology, wherein it was observed that tipulid flies comprise a large

404   proportion of the diet of *Tetragnatha* on Maui (Binford 2001, Blackledge et al. 2003). Because

405   tipulid larvae often feed on decomposing leaves (Williams 1942), it is reasonable to surmise that

406   tipulids' own δ^{13}C values fall close to those of the leaf litter, and that spiders on Hawai'i Island

407   become relatively enriched in their δ^{13}C composition when feeding on these insects.

408

409   ## Link between ecosystem properties and evolutionary processes

410   This study demonstrates that organisms at multiple tropic levels reflect the stoichiometric

411   changes in soil across the geological chronosequence of the island, from very young (200-750

412   y) to older (20,000 y). The importance of this result is that it shows that the evolutionary

413   processes associated with diversification are intimately linked to a landscape that, itself,

414   changes through time. The detailed work of Vitousek et al. (1989, 1995, 1997, 2009) documents

415   the pattern of change in soil chemistry over extended time periods: Nitrogen and phosphorus

416   increase almost linearly with time in the early stages of substrate development (up to 20,000 y

417   for nitrogen and 150,000 y for phosphorus); this increase then levels off and declines on the

418   oldest islands (4 my).

419

420   At the same time, it is now well established that organismal diversity increases over time during

421   the early stages of formation of an island archipelago (Whittaker 2008, Lim & Marshall 2017),

422   and that higher trophic levels depend on lower levels in island community assembly (Simberloff

423   & Wilson 1970), yet explanations for such patterns have, as yet, considered only area and age

424   of the landscape. The documentation of peaks of diversity on middle aged islands of the

425   Hawaiian Archipelago has been explained variously based on the interaction between age and

426   area (Gillespie & Baldwin 2010, Lim and Marshall 2017). Notably missing from these studies is

427   a link between evolutionary processes of diversification and shifts in nutrient availability

428   associated with ecosystem succession. That organisms in Hawaii are intimately reflective of the

429   ecosystem properties of their immediate habitat demonstrates that changes in nutrients

430   associated with the island chronosequence are propagated through trophic and functional

431   groups of entire biological communities. While initial work has begun to address the ecosystem

432   consequences of evolutionary change (Elser et al. 2003, Laiolo et al. 2015), this study provides

433   preliminary insights into how ecosystem change may affect processes of evolution.

434 Conclusions

435   Variation in δ^{15}N data indicates that different spider lineages reflect their different functional

436   roles and trophic positions in Hawaiian food webs, from those feeding largely on primary

437   consumers to those feeding exclusively on secondary and higher consumers. Importantly, the

438   relationships between these groups, in terms of their δ^{15}N, remain strong across the

439   chronosequence. Not only do the spiders' relative values of δ^{15}N show the same pattern at each

440   site, but their isotopic signatures also reflect the availability of nitrogen at different sites from

441   younger to older ecosystems. The tight relationship between N availability, plant isotopic values,

442   and spider isotopic values strongly suggests that the spiders are dispersal-limited and their

443   populations are isolated from one another, even across short distances. Such isolation may be

444   an important mechanism of speciation within the *Tetragnatha* adaptive radiation. This study

445   shows that these evolving lineages of spiders are intimately associated with the properties of

446   their ecosystem, which is also changing. The tight connection between the organisms and the

447   characteristics of their substrate highlights the importance of considering the role of soil

448   properties, particularly chemistry, in addition to age and area, to understand how biodiversity

449   accumulates over time.

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598

2-way ANOVA results.

Results of 2-way ANOVA testing for effects of site, functional group, and site × functional group interaction on stable isotopes of samples. Significant effects are indicated in bold.

Isotope	Effect	F	df	p-value
$\delta^{15}N$	site	692.1	2	**< 0.001**
	functional group	113.6	4	**< 0.001**
	site:functional group	8.615	6	**< 0.001**
$\delta^{13}C$	site	55.51	2	**< 0.001**
	functional group	95.15	4	**< 0.001**
	site:functional group	1.841	6	0.092

Main effects of site within functional group.

Main effects of site (substrate ages: Upper Waiakea, 200-750 y; 'Ola'a, 2,100 y; Laupāhoehoe, 20,000 y) within functional group. Site was found to have a significant effect on both C and N isotope ratios of every functional group, with the exception of $\delta^{13}C$ in plants.

Isotope	Comparison	F	df	p-value
$\delta^{15}N$	plants	78.74	2	**< 0.001**
	Spiny Leg	446.9	2	**< 0.001**
	web-builders	216.6	2	**< 0.001**
	Ariamnes	80.87	2	**< 0.001**
$\delta^{13}C$	plants	0.7997	2	0.482
	Spiny Leg	5.681	2	**0.005**
	web-builders	31.91	2	**< 0.001**
	Ariamnes	36.62	2	**< 0.001**

Main effects of functional group within site.

Main effects of functional group within site. Functional groups were found to differ significantly from one another in their isotope ratios of both C and N at all three sites.

Isotope	Site	F	df	p-value
$\delta^{15}N$	Upper Waiakea (200-750 y)	68.38	4	**< 0.001**
	'Ola'a (2,100 y)	34.23	3	**< 0.001**
	Laupāhoehoe (20,000 y)	28.90	3	**< 0.001**
$\delta^{13}C$	Upper Waiakea (200-750 y)	36.42	4	**< 0.001**
	'Ola'a (2,100 y)	41.29	3	**< 0.001**
	Laupāhoehoe (20,000 y)	41.48	3	**< 0.001**

Figure 1

Map showing field sites where samples were collected.

Locations of sites used in this study. Colors represent geology and lava flow age, determined by United States Geological Survey (Sherrod et al. 2007). Where applicable, substrate age classifications determined by the Long Substrate Age Gradient (LSAG, Crews et al. 1995) are included.
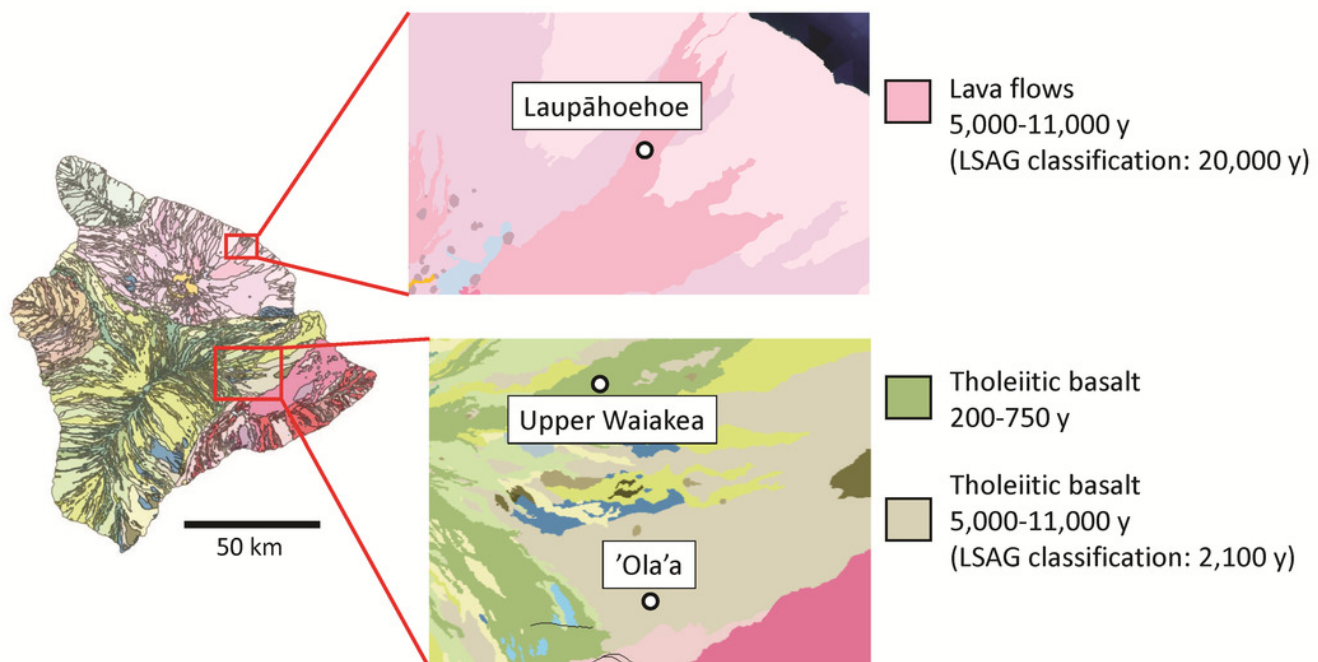
Figure 2

Boxplots showing nitrogen isotope ratios of functional groups across sites.

Nitrogen isotope ratio (δ^{15}N in ‰ units) of plant leaves (green), leaf litter (purple), Spiny Leg (brown), web-building (yellow) and Ariamnes (blue) spiders across sites of different ages.
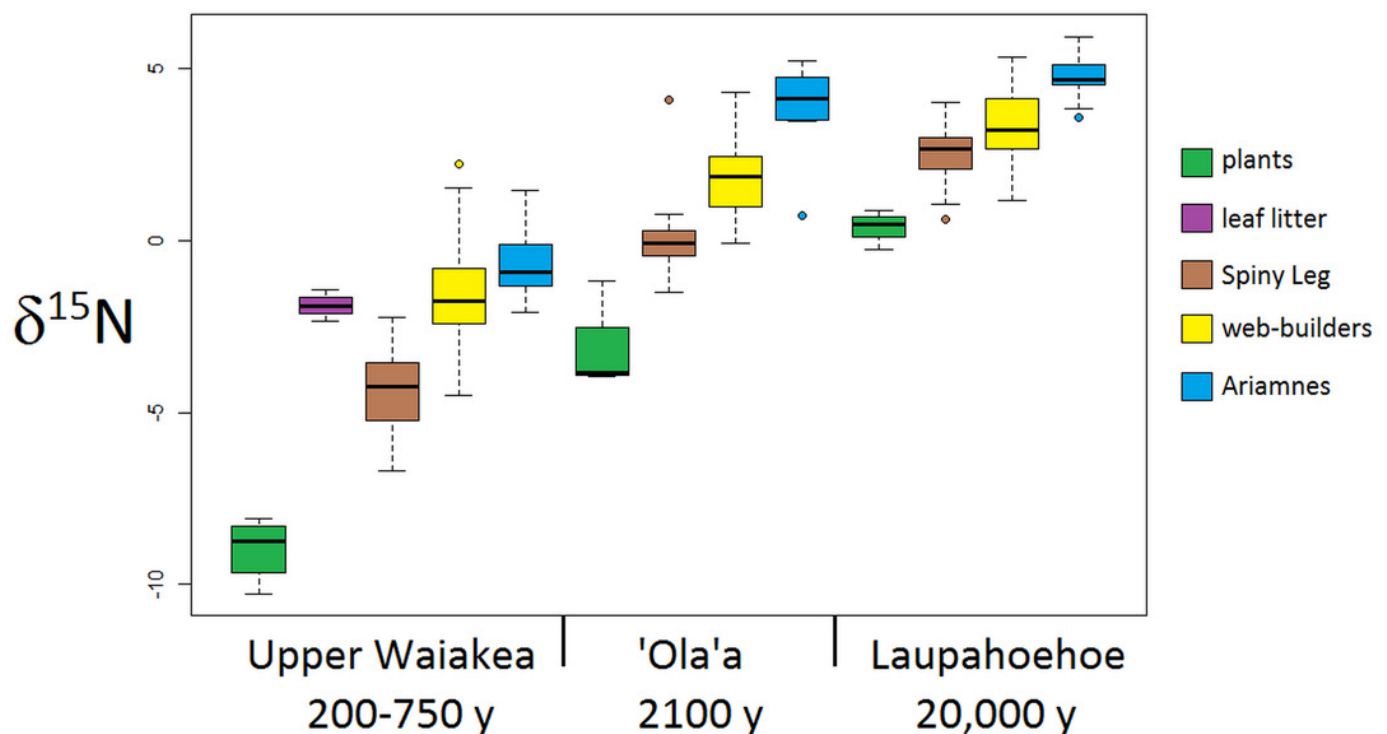
Figure 3

Boxplots showing carbon isotope ratios of functional groups across sites.

Carbon isotope ratio (δ^{13}C in ‰ units) of plant leaves (green), leaf litter (purple), Spiny Leg (brown), web-building (yellow) and Ariamnes (blue) spiders across sites of different ages.