

# Of islands on islands: A natural experiment reveals the pronounced effect of habitat fragmentation on arthropod community assembly in Hawai'i

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## Abstract

The term ‘habitat fragmentation’ is frequently associated with the biologically-destructive activities of human development, but an important evolutionary hypothesis posits that much of the biodiversity we see today was generated by episodic, natural habitat fragmentation. This hypothesis suggests that fragmentation can serve as a ‘crucible of evolution’ through the amplifying feedbacks of colonization, extinction, adaptation, and speciation. Interrogating the generality of this hypothesis requires measuring the repercussions of fragmentation at intra- and interspecific levels across entire communities. We use DNA metabarcoding to capture these repercussions from the scales of intraspecific differentiation to community composition in a megadiverse, ecologically foundational group, arthropods, using a natural habitat fragmentation experiment on patches of wet forest isolated by contemporary Hawaiian lava flows (kīpuka). We find a pronounced effect of area in kīpuka cores, where the taxonomic richness supported by a kīpuka scales with its size. Kīpuka cores exhibit higher intra- and interspecific turnover over space than continuous forest. Additionally, open lava, kīpuka edges, and the cores of small kīpuka (which are essentially entirely “edge”) host lower richness, are more biologically homogeneous, and have higher proportions of non-native taxa than kīpuka cores. Our work shows how habitat fragmentation isolates entire communities of habitat specialists, paving the way for genetic differentiation. Parsing the extent to which differentiation in kīpuka is driven by local adaptation versus drift provides a promising future avenue for understanding how fragmentation, and the different isolated communities created through this process, may lead to speciation in this system.

Running title: Hawaiian arthropods and habitat fragmentation

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## Abstract

The term ‘habitat fragmentation’ is frequently associated with the biologically-destructive activities of human development, but an important evolutionary hypothesis posits that much of the biodiversity we see today was generated by episodic, natural habitat fragmentation. This hypothesis suggests that fragmentation can serve as a ‘crucible of evolution’ through the amplifying feedbacks of colonization, extinction, adaptation, and speciation. Interrogating the generality of this hypothesis requires measuring the repercussions of fragmentation at intra- and interspecific levels across entire communities. We use DNA metabarcoding to capture these repercussions from the scales of intraspecific differentiation to community composition in a megadiverse, ecologically foundational group, arthropods, using a natural habitat fragmentation experiment on patches of wet forest isolated by contemporary Hawaiian lava flows (kīpuka). We find a pronounced effect of area in kīpuka cores, where the taxonomic richness supported by a kīpuka scales with its size. Kīpuka cores exhibit higher intra- and interspecific turnover over space than continuous forest. Additionally, open lava, kīpuka edges, and the cores of small kīpuka (which are essentially entirely “edge”) host lower richness, are more biologically homogeneous, and have higher proportions of non-native taxa than kīpuka cores. Our work shows how habitat fragmentation isolates entire communities of habitat specialists, paving the way for genetic differentiation. Parsing the extent to which differentiation in kīpuka is driven by local adaptation versus drift provides a promising future avenue for understanding how fragmentation, and the different isolated communities created through this process, may lead to speciation in this system. Translated abstracts are available in the supplementary information.

## Key words

kīpuka, metabarcoding, biodiversity, non-native species, genetic differentiation, natural fragmentation

## Introduction

Habitat fragmentation plays a fundamental role both in shaping biological communities and in intraspecific diversification. As barriers to migration increase, or as population sizes decrease, the risk of overall extinction of a taxon increases (Hanski 1989, Hanski & Gilpin 1991, Reed 2004). Moreover, the isolating matrix itself may facilitate colonization from outside the system, by species better able to survive in the new conditions created (Cacabelos et al. 2022, Hobbs 2001). However, in some situations, the isolation created by fragmentation can lead to species undergoing local adaptation (White et al. 2022), and potentially even speciation when fragments merge after longer time periods (Flantua et al. 2019). The latter situation is a key premise in the theory that much of the evolution and diversification of life has been driven by the coevolution of species with their ever-changing ecological communities (the mosaic theory of coevolution; Thompson 2005), and the concept of constantly changing (evolving) metacommunities (Urban & Skelly 2006). However, because fragmentation changes the diversity and composition of entire biological communities, establishing very different selective regimes in the different fragments (Kuli-Révész et al. 2021), there are multiple feedbacks that affect the interplay of colonization and extinction versus adaptation and speciation (De Meester et al. 2016). Only through study at the scale of entire communities can we start to develop insights into the role of fragmentation in restructuring local communities on one hand and differentiation on the other (Leibold et al. 2022).

Such large-scale analyses have long been limited by the sheer taxonomic diversity of studied communities. Recent developments in high throughput DNA barcoding now allow for the rapid inventory of entire com-

munities at multiple levels, from taxonomic composition to intraspecific genetic variation (Arribas et al. 2022). This opens up unprecedented opportunities to study community assembly in response to environmental differences and habitat isolation (Emerson et al. 2022). These techniques have been particularly useful in the study of arthropods, the most species-rich animal taxon. Though arthropods are compelling to study because of their ecological diversity and their centrality to the food web of most terrestrial ecosystems, their overwhelming taxonomic diversity makes the manual classification of community samples impractical and means that this taxon is particularly underdescribed (Emerson et al. 2016).

The current study couples a unique natural fragmentation experiment on the Hawaiian Islands with a novel bulk community metabarcoding approach to infer how entire arthropod communities change across discrete spatial scales in a fragmented landscape, both in terms of species composition and intraspecific differentiation. The Hawaiian Islands are ideal for examining the effects of isolation: The archipelago itself is the most isolated in the world, which means that there were very few successful colonists (Wagner and Funk 1995). Within the archipelago, each island is isolated, with many of the endemic lineages showing a pattern of progression that follows the geological chronology of the islands from older to younger (Shaw & Gillespie 2016). Within an island, isolation is created by separation of volcanoes and, over the most recent time scales, new lava flows that can carve up the existing forests (Vandergast and Gillespie 2004, Vandergast et al. 2004). In the latter case, lava flows from volcanic eruptions lead to isolation of forest fragments for extended time periods, with forest regrowth on lava substrate taking anywhere from 300 to 3,000 years depending on the abiotic environment (Drake & Mueller-Dombois 1993, Clarkson 1998). The early history of each island has been characterized by perpetual cycles of such dramatic fragmentation (Carson et al. 1990). The effects of these cycles can be observed in real time on the youngest island of Hawai'i, where lava from active volcanoes has led to multiple episodes of forest fragmentation. This pattern is striking on the slopes of Mauna Loa Volcano, where flows have repeatedly covered the landscape over the last 400,000 years, creating a patchwork of habitats in different successional stages (Carson 1995). Here, mature forests have often been surrounded by new flows, resulting in patches of forested "islands" (kīpuka) of various sizes and distances from each other and from the continuous forest. The contrast between forest and lava is stark, with the forest patches being continuously wet and cool, while the surrounding lava is dry and hot during the day and cold at night. Hawaiian forest arthropods are well known for their narrow climatic niches (Hiller et al. 2019, Lim et al. 2022) and hence expected to be strongly isolated by the inhospitable lava (Vandergast et al. 2004, Vandergast & Gillespie 2004).

A particularly well-studied kīpuka system is located along the northwestern slopes of Mauna Loa, where a volcanic eruption in 1855 has left a network of multiple forest patches separated by barren lava. Studies in this location have provided evidence that kīpuka differ in community composition (Knowlton et al. 2017, Mueller 2015), and that arthropods that are specialized for the wet forest are largely isolated in a kīpuka, with the isolation sufficient to allow populations in different kīpuka to demonstrate genetic differentiation (Carson & Sato 1969, Vandergast et al. 2004).

It has long been speculated that a kīpuka-like landscape dynamic, fostering repeated isolation events, may serve as a "crucible of evolution" (Carson 1990). According to this hypothesis, the isolation of populations by lava flows has played a key role in fueling adaptive radiation on the Hawaiian Islands. At the same time, it provides a natural experiment for testing how fragmentation affects the species composition of habitat patches of different size and isolation; not only are kīpuka sometimes very small, so unlikely to sustain a high diversity of forest-specialists over time. This can be exacerbated by a substantial "edge" of low native species diversity that penetrates an estimated 20 m-deep into kīpuka forest (Vandergast & Gillespie 2004). The declines in species diversity towards kīpuka edges and with declining kīpuka size can make these fragments more prone to colonization from non-indigenous species (Mueller 2015).

Here, we use the Mauna Loa kīpuka system to examine how entire communities of arthropods have responded to habitat fragmentation over a 170 year period. We use a vegetation beating protocol (Lim et al. 2022) to sample forest undergrowth arthropod communities at the core and edge of 13 kīpuka of varying sizes and distances to each other. As a comparison to the kīpuka, we sampled two transects in continuous forests on

the eastern and western slopes of Mauna Loa. We applied a semiquantitative metabarcoding protocol, which allowed us to study communities from the scale of taxonomic diversity to the scale of intraspecific genetic differentiation. We hypothesize that

- 1) Due to the pronounced climatic specialization of Hawaiian forest arthropods, lava will have presented a strong barrier to their dispersal, resulting in a community-wide species-area effect. Specifically, we expect smaller kīpuka to have lower taxonomic and genetic diversity. We expect to see higher community turnover over similar distances between kīpuka than between continuous forest sites. For the same reason, species that are limited to the forest core of a kīpuka will, on average, show more evidence for genetic differentiation than species sampled across similar distances in continuous forest.
- 2) Given the environmental differences between forest at the core of the kīpuka compared to the edges (Vandergast & Gillespie 2004), we expect markedly different arthropod species composition between them. In individual kīpuka, we expect forest-specialized taxa to dominate core habitats. Meanwhile, we expect that fewer forest species will persist in kīpuka edges, and that both edge habitats and adjacent lava matrix will have been colonized by higher proportions of non-native taxa than kīpuka cores or native forest habitats. Consequently, kīpuka should host distinct communities in their cores versus their edges, excepting in the case of the smallest kīpuka, which may behave almost entirely as edges.
- 3) The species-area effect of reduced species diversity with decreasing kīpuka size should provide less biotic resistance, resulting in an inverse relationship between kīpuka size and the proportion of non-native taxa detected in both core and edge habitats.

## Materials and methods

### Sampling

We sampled the arthropod community in continuous and fragmented wet forest communities at different locations across the slopes of the Mauna Loa volcano on the Big Island of Hawai'i (Fig. 1). Sites were at approximately the same elevation, with similar precipitation, and all were dominated by the canopy tree *Metrosideros polymorpha*. Undergrowth was dominated by *Cybotium glaucum* fern and other native plants, with minimal impact of invasive plant species. We took GPS coordinates from each site and acquired data for geographic distances between sites. Kīpuka areas were measured in GIS by calculating the area of polygons manually drawn around kīpuka margins in satellite images of the study landscape.

The two continuous forest areas were sampled in August 2019. On the windward side of the mountain, we sampled 12 sites in the Waiakea Forest Reserve, hereafter referred to as Stainback, from 876 – 1,494 m a.s.l.. The Stainback sites receive 2,767 – 6,126 mm of average annual precipitation, and their annual temperatures range from 13.45 – 16.84 °C. The sites were situated 333–11,001 m from one another, with an average distance of 5,437 m. On the leeward side of Moana Loa, we sampled 10 sites from 1,058 – 1,455 m a.s.l. in the Kona Hema Forest Reserve. Kona Hema sites receive 803-1,005 mm of average annual precipitation, with annual temperature ranging from 14.21 – 16.74 °C. These sites were 349 – 3,256 m away from one another, with their average distance being 1,652 m.

In addition, we sampled 13 kīpuka on Mauna Loa, which resulted from a volcanic eruption in 1855. The kīpuka system is a mid-elevation area in a relatively remote area of the island, in which the forest is almost entirely native (Wagner 1999). Forest fragments, previously connected by continuous forest (Lockwood et al. 1988), were isolated from one another by an 1855 lava flow from Mauna Loa Volcano (Fig. 1; map coordinates: N 19°37'40" and W 155°21'15"). Elevation at this area ranges from 1,524 – 1,646 m, annual temperature from 12.00 – 13.25 °C, and annual precipitation from 2,279 – 2,765 mm. The position of the kīpuka in a very similar climate belt controls for the effects of climate and makes this an ideal system to study the effect of geographic isolation on communities. The forested area of the sampled kīpuka ranged

from 555 – 100,081 m<sup>2</sup>, and the average size was 23,447 m<sup>2</sup>. The distance between kīpuka ranged from 49 m – 4,744 m, and the average distance was 1,766 m.

To compare arthropod communities in the dense forest in the kīpuka center with the more open edges, we sampled them separately. At each kīpuka, an edge sample was collected approximately 10 m into the forested area. The kīpuka are surrounded by mostly continuous lava, which is very hot and dry compared to the forest vegetation in the kīpuka. To explore which arthropods can easily cross this hostile habitat, we collected at four sites in the lava matrix surrounding the sampled kīpuka, in which vegetation of stunted and isolated *Metrosideros polymorpha* trees was found.

Arthropods were collected from each site using beat sampling, according to the approach described in Lim et al. 2022. Beat sampling of all undergrowth vegetation was performed in a 10 m radius around the centroid of the forest plot for two person hours. All arthropod specimens were collected from the beat sheets, stored in 99 % ethanol, and transported back to University of Trier in Germany.

#### Specimen size sorting, DNA extraction, PCR and sequencing

We processed all samples using a semiquantitative metabarcoding protocol as described in Lim et al. 2022. Large arthropod specimens contribute considerably more biomass and hence DNA to a sample than small ones, so to reduce biases by large arthropod specimens in the sample, we sorted all specimens into four size categories aimed at approximately distributing biomass: 0 – 2 mm, 2 – 4 mm, 4 – 7 mm, and > 7 mm. One particularly abundant arthropod group in Hawaiian wet forest is Collembola. We thus sorted Collembola out as a fifth category. All size sorted samples were stored in 2 ml tubes. After addition of a five mm stainless steel ball, lysis buffer and proteinase K, specimens were finely ground on a Qiagen Tissuelyzer (Qiagen, Hilden, Germany) and then DNA was extracted from each size sorted sample using the Qiagen Puregen kit according to the manufacturer’s protocol.

A 365 bp fragment of the mitochondrial COI gene was then amplified separately for each sample and size category using the primer pair mlCOIintF/HCOdeg (Leray et al. 2013, Yu et al. 2012). PCRs were run using the Qiagen Multiplex PCR Kit, according to the manufacturer’s protocol, with 32 cycles and at an annealing temperature of 46 °C. PCR success was checked using agarose gel electrophoresis. After the first round of PCR, an indexing PCR was performed, with five cycles and an annealing temperature of 55 °C, in which Illumina Truseq adapters and indexes were incorporated on each fragment (Lange et al. 2014). The resulting libraries were quantified using agarose gel electrophoresis and pooled in approximately equimolar amounts. The final sample was purified using 1 X Ampure Beads XP (Beckman Coulter, Brea, CA, USA) and sequenced on an Illumina MiSeq using V3 chemistry and 600 cycles (Illumina, San Diego, CA, USA). No-template control PCRs and negative control extraction samples were also amplified and sequenced alongside the arthropod samples.

#### Sequence analysis

The resulting reads were demultiplexed with zero mismatches allowed and then merged using PEAR (Zhang et al. 2014) with a minimum overlap of 50 bp and a minimum quality of 20. The merged read pairs were additionally quality filtered for a minimum of 90 % of bases at a quality of [?] 30 and transformed to fasta format using the fastx toolkit (Gordon & Hannon 2010). PCR primers were trimmed and all separate samples were merged into a combined file. USEARCH (Edgar 2010 & 2016) was used to dereplicate the sequences and cluster them into zero radius OTUs (zOTUS) using the *unoise3* command and 3 % radius OTUs (OTUs) using the *cluster\_otus* command. While OTUs should approximate species in the dataset, zOTUs represent haplotypic variation in individual species. Using BLASTn (Altschul et al. 1990), we linked OTUs and matching zOTU. Each 3 % radius OTU should consist of [?] 1 zOTUs, with a maximum dissimilarity of 97 %. We then assigned taxonomy to all resulting OTUs and their matching zOTUs using BLASTn against the complete GenBank database (downloaded 02/2021) with a maximum number of 10 matches. We only retained sequences identified as arthropods and assigned order status to them. We refrained from annotations to lower taxonomic levels, due to the incomplete barcode reference libraries for most Hawaiian arthropods. Spiders (order Araneae) are a notable exception, with well-developed reference libraries. The majority of

spiders could be classified to species level. The resulting, well-resolved taxonomic information for spiders was used to classify OTUs into native and non-native species in Hawai'i. Using spiders as a model taxon, we could thus test the resilience of different habitat types against biological invasions.

A zOTU table was created using the *otutab* command in USEARCH. The resulting table consisted of five entries for each sample, e.g., the four size categories and the Collembola sample. We then used the number of specimens in each of the five categories, to rarefy this table and merge the size categories into one final sample according to Lim et al. (2022). Briefly, the total read number for each category was subsampled by the number of specimens in that category. Each sampled specimen was represented by 15 reads in the final table. After rarefaction, the five categories were merged into final counts for each site. Using this final table, we calculated patterns of alpha and beta diversity for zOTUs and 3 % radius OTUs in *vegan* (Oksanen et al. 2007) in R (R Core Team 2021).

## Results

### Alpha diversity and invasion success in kīpuka centers, edges, and continuous forest habitat

We collected a total of 38,289 individual arthropod specimens overall (Stainback: 11,610, Kona Hema: 12,130; kīpuka center: 7,887; kīpuka edge: 5,768; lava: 894). Different numbers of specimens were found for the different size categories (0 – 2: 11,653 total, 224 average; 2 – 4: 5,532 total, 106 average; 4 – 7: 1,917 total, 37 average; >7: 1,713 total, 33 average; Collembola: 17,474 total, 336 average). These specimens represented a total of 923 arthropod OTUs and 3,022 zOTUs. The zOTUs belonged to 23 orders, of which the most diverse were Hemiptera (675), Araneae (423), Diptera (337), Lepidoptera (299) and Coleoptera (249).

Different areas showed relatively comparable order compositions. This held true for relative zOTU read abundances (Suppl. Fig. 1A) as well as zOTU richness (Suppl. Fig. 1B). However, a clear difference was evident when comparing lava and kīpuka edges with the remaining areas, specifically within the orders Coleoptera and Diptera: lava and edges were distinguished by significantly fewer reads and zOTUs.

The continuous forests of Kona Hema and Stainbeck hosted similar arthropod richnesses, but a significantly higher arthropod richness than kīpuka sites (Fig. 2A). Moreover, we found a significantly higher richness in kīpuka cores than in kīpuka edge habitats. An even lower richness was found in barren lava (average zOTU|OTU richness Stainback: 332|145; Kona Hema: 331|152; kīpuka center: 246|120; kīpuka edges: 207|101; lava: 116|56). These general trends also largely held up when separate orders were analyzed (Suppl. Fig. 2). However, for Diptera, kīpuka centers showed comparable richness to continuous forest sites; for Psocoptera, all sites showed comparable richness; and, for Hemiptera, only lava sites were less species rich.

We found a significant positive correlation of logarithmic kīpuka size and the recovered zOTU and 3 % radius OTU richness in kīpuka centers (Fig. 2B;  $r^2_{\text{zOTU}} = 0.54$ ,  $r^2_{\text{OTU}} = 0.47$ , LM  $p < 0.05$ ). Larger kīpuka harbored a significantly more diverse community in their center than smaller ones. The increase of richness with area was steeper for zOTU, increasing by 79 % from 165 to 295 zOTUs over kīpuka sampled, than it was for 3 % radius OTUs, where it increased only 31 % from 86 to 125. Small kīpuka showed a considerably lower 3 % radius OTU and zOTU richness than continuous forests. However, large kīpuka approached richness values comparable to those documented for continuous forest, with richness approaching saturation in the cores of the seven largest kīpuka (7,388 m<sup>2</sup> – 100,081 m<sup>2</sup>), particularly for OTUs. In contrast to kīpuka centers, we did not find an association of zOTU or 3 % radius OTU richness with kīpuka area for edge habitats (Fig. 2B;  $r^2_{\text{zOTU}} = 0.07$ ,  $r^2_{\text{OTU}} = 0.12$ , LM  $p > 0.05$ ). The richness-area relationship for kīpuka centers could also be replicated when separate orders were analyzed (Suppl. Fig. 3). It was particularly strong for Coleoptera ( $r^2 = 0.48$ ) and Diptera ( $r^2 = 0.73$ ). Araneae was the only order for which richness and kīpuka area were significantly associated in edge habitat ( $r^2 = 0.38$ ; LM  $p < 0.05$ ).

While we found an overall higher spider species richness in continuous forest habitats than in kīpuka (Suppl.

Fig. 2A), an inverse pattern was found for non-native spider species (Fig. 3A). The highest proportion of reads of non-native spiders was found in barren lava and kīpuka edge habitat. Significantly lower proportions of non-native spider reads were found in continuous forest and in kīpuka centers, with no significant differences between kīpuka centers and continuous forests. Kīpuka centers harbored a significantly lower proportion of non-native spiders than edges (Fig. 3A).

A strong species-area effect was also found for the proportion of non-native spider reads. The proportion of non-native spider OTUs had a highly significant, negative association with logarithmic kīpuka size (Fig. 3B). Large kīpuka harbored a considerably lower proportion of non-native spider reads and OTUs than small ones. A particularly strong drop of invasion success was visible between the four smallest (555 – 1,619 m<sup>2</sup>) and the remaining nine kīpuka (4,212 – 100,081 m<sup>2</sup>). This effect was visible in kīpuka centers and edges, but was particularly pronounced for edge habitat, which generally showed a higher presence of non-natives.

#### Beta diversity and kīpuka size

NMDS plots of zOTUs and OTUs showed a clear separation of the arthropod community composition in our different sites (Fig. 4A & C). The first axis separated the continuous forests from kīpuka, while the two continuous forests in Stainback and Kona Hema were well-separated by the second axis. The NMDS plots also showed a separation of kīpuka center and edge habitats and barren lava. The differentiation of center and edge habitat was particularly pronounced in larger kīpuka. While the five smallest kīpuka (area 555 – 4,212 m<sup>2</sup>) grouped closer to the edge samples, the remaining eight (area 5,458 – 100,081 m<sup>2</sup>) were clearly distinct and formed a separate cluster. In comparison to sites in each continuous forest area, kīpuka were considerably more dispersed in the NMDS plots. Moreover, sites in kīpuka centers grouped closer to continuous forest sites than to the edge sites, suggesting that kīpuka center communities are more similar to continuous forest communities. While zOTU and 3 % radius OTU based NMDS supported similar patterns of differentiation, the differentiation between the edges and centers of large kīpuka was more pronounced for the 3 % radius OTU dataset.

The separation of different areas shown in the NMDS plot was also supported by patterns of beta diversity for zOTUs and 3 % radius OTUs (Fig. 4B & D). With a notable exception of sites in barren lava and the center of kīpuka, which showed the highest dissimilarity, the average within-area dissimilarity (average zOTU|OTU beta Stainback: 0.57|0.55; Kona Hema: 0.60|0.58; kīpuka center: 0.78|0.76; kīpuka edges: 0.69|0.64; Lava: 0.76|0.72) was significantly lower than between-area comparisons (average zOTU|OTU beta Stainback-Kona: 0.85|0.79; Stainback-center: 0.86|0.83; Stainback-edge: 0.85|0.83; Stainback-lava: 0.92|0.91; Kona-center: 0.92|0.88; Kona-edge: 0.91|0.88; Kona-lava: 0.95|0.94; center-edge: 0.79|0.76; center-lava: 0.86|0.84; edge-lava: 0.76|0.70). Within-area dissimilarity in continuous forest was significantly lower than in kīpuka habitats. This held true particularly for kīpuka centers, which showed a higher within-area differentiation than edges. Hence, the edges of different kīpuka were more connected than their corresponding centers. These patterns held true for zOTUs and 3 % radius OTUs, but the difference between kīpuka and continuous forest areas was slightly higher for zOTUs. The higher within-area differentiation of kīpuka centers compared to continuous forest and kīpuka edge held up even when only the eight largest kīpuka, which formed a separate cluster closer to continuous forest on the NMDS plot, were compared to each other (average zOTU|OTU beta Stainback: 0.59|0.55; Kona Hema: 0.61|0.58; kīpuka Center: 0.79|0.75; kīpuka Edges: 0.71|0.66; Lava: 0.79|0.72).

Centers and edges of the same kīpuka harbored distinct arthropod communities, particularly for larger kīpuka (> 5,000 m<sup>2</sup>; Suppl. Fig. 4). In fact, the dissimilarity between the center and edge of each kīpuka showed a pronounced association with the area of that kīpuka. Community dissimilarity between centers and edges steadily increased with kīpuka size for small kīpuka, but began saturating for kīpuka larger than 5,000 m<sup>2</sup>.

Our previous NMDS analysis revealed two separate communities in kīpuka centers: kīpuka so small they behaved more like kīpuka edges (< 5,000 m<sup>2</sup>) and larger kīpuka with core communities more similar to continuous forest communities (> 5,000 m<sup>2</sup>). To explore the association of beta diversity with distance between sites, we thus generated two kīpuka datasets. The first dataset included all kīpuka, the second consisted of only the eight largest kīpuka (5,458 – 100,081 m<sup>2</sup>), which formed a well-separated cluster from

edge habitats in our NMDS plots, and the third one consisted of the five smallest kīpuka (555 – 4,212 m<sup>2</sup>). An analysis of within-area beta diversity for these datasets showed a significantly higher community dissimilarity between centers of large kīpuka than those of small kīpuka. At the same time, no significant difference of community dissimilarity for edges of large and small kīpuka was found.

We next analyzed patterns of community isolation by distance for continuous forests, kīpuka centers and edges. The continuous forests both showed a significant and quite similar linear association of logarithmic geographic distance and community dissimilarity (Fig. 5;  $r^2_{\text{Kona Hema}} = 0.16$ ;  $r^2_{\text{Stainback}} = 0.74$ ; LM  $p < 0.05$ ). We also found a positive linear association of beta diversity and logarithmic geographic distance for the kīpuka sites (Fig. 5;  $r^2_{\text{center}} = 0.12$ ;  $r^2_{\text{edge}} = 0.30$ ; LM  $p < 0.05$ ). Compared to continuous forest, the overall dissimilarity was much higher for kīpuka, even at short geographic distances. This pattern was particularly strong for kīpuka centers, while kīpuka edge habitats appeared to be more connected. Community dissimilarity between kīpuka centers saturated at very low geographic distances. The community differentiation between kīpuka centers isolated by only a few 100 m of barren lava was comparable to the differentiation between continuous forest sites isolated by over 10 km. These patterns also held up when different arthropod orders were analyzed separately (Suppl. Fig. 5 & 6). Nearly all orders showed a saturated or nearly saturated beta diversity with distance for kīpuka centers, while an association with geographic distance was found for edge habitat. Diptera was the only order for which both center and edge showed similar patterns of isolation by distance.

## Discussion

Our primary goal was to assess the interplay between extinction-recolonization dynamics on the one hand, versus isolation and local adaptation on the other, in shaping the dynamics of arthropod communities across a naturally fragmented landscape. Using metabarcoding of whole arthropod communities in kīpuka of different size, we found that forest-specialist arthropods have been isolated by kīpuka fragmentation to the extent that we can detect that they have genetically differentiated over a 150 year timescale. However, we simultaneously used this dataset to demonstrate that species distributed across the kīpuka system have differentiated in entirely different biological communities from one another— suggesting how kīpuka might drive local adaptation to different biotic environments.

### Kīpuka as crucibles of evolution

Our data provided strong evidence for our first hypothesis, that we would document a species-area effect relating to community-wide isolation. Lava flows pose a strong dispersal barrier to core forest arthropods (Roderick et al. 2012, Carson et al. 1990), so we did recover a strong species-area effect consistent with previous results from spiders (Vandergast et al 2004), *Drosophila* flies (Mueller 2015), birds (Flaspohler et al. 2010), and root fungi associated with *M. polymorpha* (Vannette et al. 2016). The isolation of communities by lava also caused considerably higher community turnover between kīpuka than between equivalently spaced sites in continuous forest. Even kīpuka isolated by only a few 100 m of lava sometimes showed nearly complete community turnover. As further predicted by our first hypothesis, the community-wide isolation between kīpuka also translated into genetic differences between populations of individual species, as has already been shown in a few spiders (Vandergast et al. 2004, Roderick et al. 2012) and flies (Mueller 2015). The regrowth of forest after volcanic eruptions on Hawai'i can take from several hundred to several thousand years, providing ample time for genetic differentiation between populations in individual kīpuka by genetic drift (Lourenço et al. 2017). Allopatric isolation, a major driver of speciation for organisms across the tree of life, can happen over long geological time periods and large geographic distances—through continental drift or the emergence of mountain ranges— but we also know that even very short geographic distances can suffice to isolate populations (Johnson & Munshi-South 2017). Our analysis suggests that fragmentation by lava flows could pose a possible means for such microallopatric differentiation for Hawaiian arthropods.

The extreme community turnover between neighboring kīpuka means that individual arthropod species are

exposed to very different communities of interacting species in different kīpuka, and hence different selective pressures. For example, a single species present in different kīpuka might interact with different competitors, predators or parasitoids. Interspecific interactions are highly important drivers of speciation (Thompson 2005), so these distinct biotic communities may accelerate the differentiation of taxa between kīpuka. Besides the possibility kīpuka provide for adaptation to different biotic environments, kīpuka may provide opportunities for adaptation along climatic gradients. For instance, adjacent kīpuka are frequently located along pronounced temperature and precipitation gradients in Hawai'i, such that the isolation of species in these kīpuka could foster phenotypic differentiation through local climate adaptation. In a continuous forest this adaptation would likely be swamped by gene flow. While here we focused on kīpuka distributed within a relatively similar climatic zone, an old kīpuka system distributed across a climatic gradient would provide an excellent setup for studying the impacts of microallopatry on rates of phenotypic differentiation. A final means by which kīpuka may foster differentiation is through the merging of formerly isolated kīpuka: admixture of the isolated populations they contain could provide the raw material for rapid evolutionary responses, as has been suggested as the underlying cause for many adaptive radiations (Marques et al. 2019).

### Kīpuka demonstrate how fragmentation affects forest communities

From our results, we find that kīpuka are, in fact, generally more sensitive to biological invasions than continuous forest. Incursions of non-native species into native forests is notoriously high on islands (Vitousek 1988), which may be associated with low phylogenetic and functional diversity (e.g. Sayol et al. 2021, Baiser et al. 2018). This effect is accentuated by fragmentation that, besides carving up native landscape, creates edge habitat within the forest patches, with both the matrix and edges associated with an increase in non-native species (As 1999). Such disturbance facilitates invasions of exotics by creating open ecological space (Meyer et al. 2021, Hobbs 2000). Previous work has suggested that the kīpuka system, being at high elevation and being contained within an otherwise in a relatively undisturbed forested area, is characterized by an arthropod fauna that is almost entirely native, with non-natives mainly affecting edge and lava areas (Vandergast & Gillespie 2004). However, we also found considerable proportions of non-native species in kīpuka cores.

In accordance with our second hypothesis, we observed a strong decline in diversity towards kīpuka edges in the present study. Many forest arthropods have narrow climatic niches (Lim et al. 2022), which appears to have limited them to climatically stable forest cores. Many forest taxa clearly avoided the open forest at kīpuka edges, which was instead occupied by higher abundances of the few edge-tolerant species. Additionally, we found significantly higher proportions of non-native taxa in edge than in core habitats, making kīpuka edge and core communities entirely distinct from one another.

In line with our third hypothesis, we found significantly more non-native taxa in the cores of smaller kīpuka. In fact, we found that small kīpuka below a size of about 5,000 m<sup>2</sup> were profoundly impacted by non-native species and had very low species richness, even in their cores. This finding may either mean that the smaller a kīpuka was, the less resilient its core forest community appeared against incursion of non-natives, or alternatively that small kīpuka behave entirely as edge where non-natives adapted to disturbed conditions are better able to colonize and persist.

### Kīpuka as a model for anthropogenic fragmentation

Kīpuka are a well-suited natural experiment we can use to explore the effect of anthropogenic habitat fragmentation on tropical forest arthropod communities. Indeed, the stark contrast in conditions provided by lava relative to moist tropical forest makes kīpuka systems an interesting model for studying urbanization and road-building. The parallels are particularly striking in terms of the abiotic conditions created by lava versus paved surfaces. Of course, we must note that the parallelism to urbanization and road-building is limited at the biotic level. Whereas lava flows are themselves sterile and merely create conditions suited to the persistence of non-natives that passively arrive to the kīpuka system, urbanization and road-building are in fact conduits for the active introduction of non-natives to the disturbed zone.

While tropical forests harbor the majority of global terrestrial biodiversity (Ghazoul & Sheil 2010), the

resident taxa appear to be extremely sensitive to the effect of habitat fragmentation, at least according to what we learn from examining Hawaiian arthropods in kīpuka. Isolation by lava flows and resulting species-area effects means that small kīpuka harbor significantly reduced species richness. Even the forest edge of kīpuka is entirely avoided by many forest-specialist species, which are dominated by a few edge-tolerant native taxa living aside non-native colonists. The near complete isolation of kīpuka communities across very short geographic distances, supported by accelerated species turnover over similar distances in the kīpuka compared to the continuous forest system, suggests that even a wide road may suffice to prevent dispersal for many arthropod taxa. Intriguingly, the species-area effect we observed in the kīpuka system was even more pronounced at the haplotype level; i.e., genetic diversity may be affected more strongly by isolation in small fragments than taxonomic diversity. Hence very small kīpuka might have less evolutionary value as the haplotype diversity they contain for any given species dwindles under powerful genetic drift, in the process accumulating high extinction debt relative to wet forest taxa. In this light, our results suggest that even a network of relatively close forest fragments might not promote the persistence of viable metapopulations of some specialized forest taxa.

## Conclusion

Within the exciting, natural fragmentation experiment provided by Hawaiian kīpuka, we have used a powerful metabarcoding approach to study the role this fragmentation can play in simultaneously restructuring biological communities and driving interspecific differentiation. We found that Hawaiian kīpuka were characterized by simpler communities with higher proportions of non-native taxa than Hawaiian continuous forest. Kīpuka communities were more distinct from one another than communities sampled across similar distances in continuous forest, and similarly we found evidence for higher rates of genetic differentiation between kīpuka than across similar distances sampled in continuous forest. Conceivably, our results suggest that forest specialist species in these ‘islands’ might undergo more granular local adaptation in kīpuka than they would otherwise do in continuous forest habitat.

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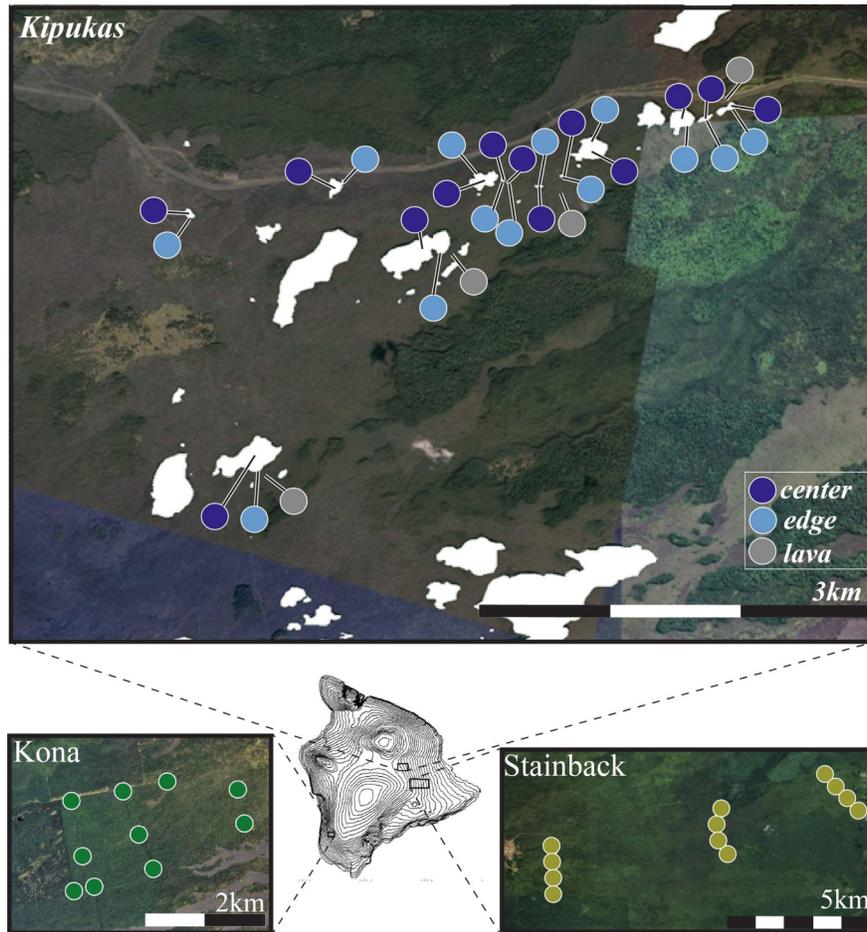
## Data accessibility and benefit-sharing

Data characterizing the sites and the zOTUs and 3 % OTUs sampled there have been deposited at DataDryad (doi: XXXX). Sequencing data is available through the NCBI Sequence Read Archives (SRA) under BioProject Accession Number XXXXXX. Code is available at <https://github.com/steigeec/Kipuka>.

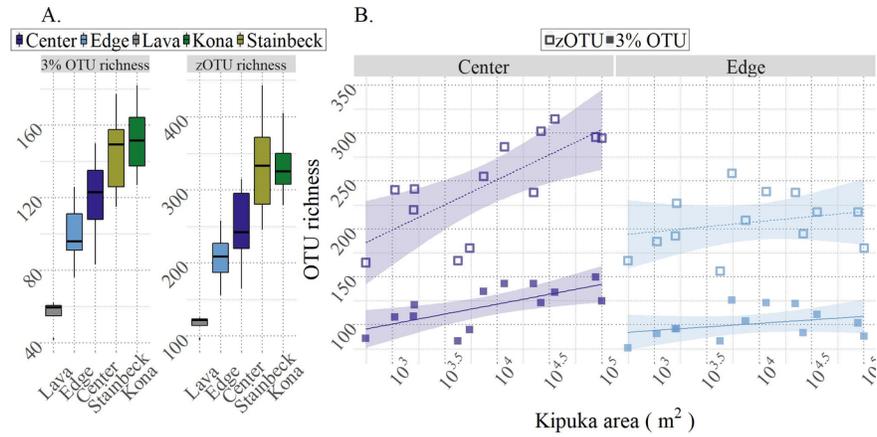
## Author contributions

All authors contributed to performing the research and writing the paper. ES and HK analyzed the data. HK designed the study.

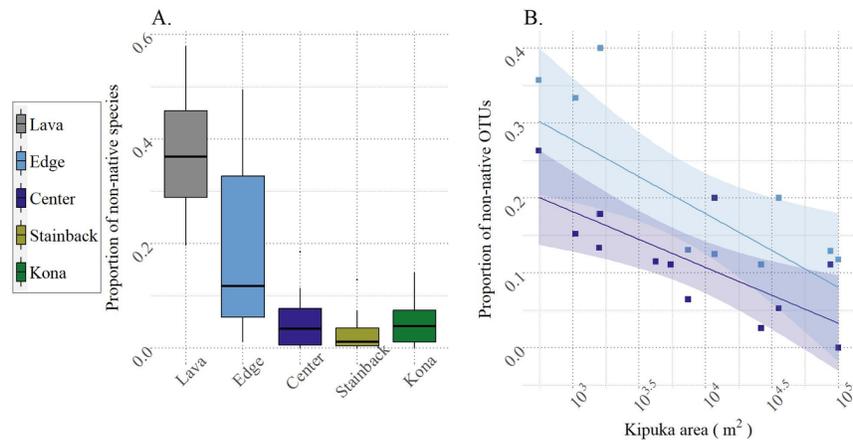
## Tables and figures



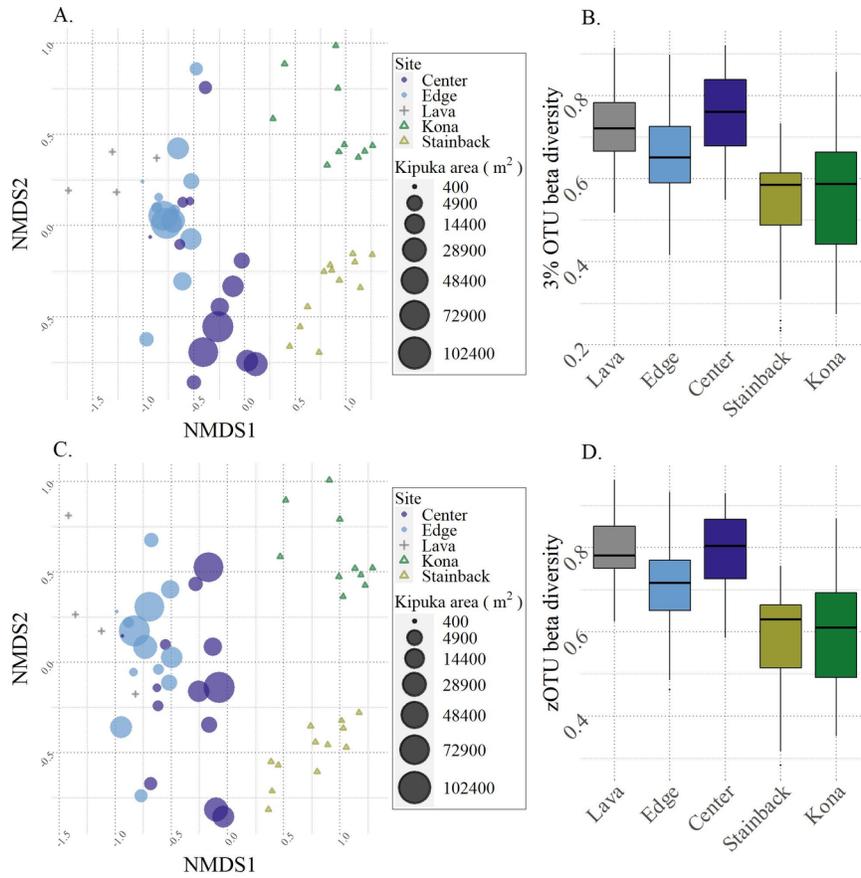
**Figure 1** Overview of sites on the slopes of Mauna Loa on the Big Island of Hawaii. We sampled a total of 13 kipuka sites, each in a paired fashion with a central and an edge site (dark blue and light blue, respectively). In addition, we sampled four sites on barren lava in the matrix connecting kipuka. As a control to the kipuka sites, we sampled at sites in two continuous forests along the western and eastern slopes of Mauna Loa (Stainback and Kona Hema, respectively).



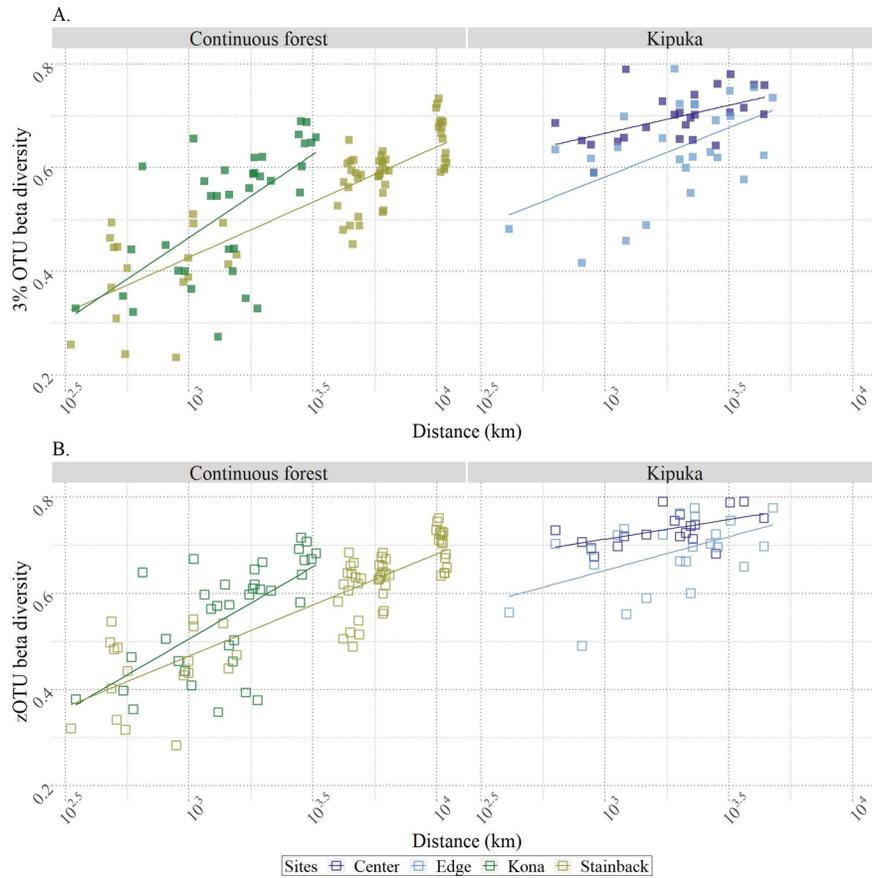
**Figure 2** Species richness for different sites. A) 3 % radius OTU richness and zero radius OTU (zOTU) richness in barren lava, kipuka edges, kipuka centers, and continuous forest in Stainback and Kona Hema. B) Effect of kipuka size on diversity of edge and center communities. The plots show kipuka area on a log scale plotted against 3 % radius OTU richness and zOTU richness, for kipuka centers on the left and edges on the right.



**Figure 3.** The relationship between site type or kipuka area and non-native species richness or read abundance in Araneae. (A) The proportion of non-native 3 % OTUs by site type. (B) kipuka size, on a log scale, versus. the proportion of non-native spider 3 % OTUs for kipuka edges ( $r^2 = 0.5451$ ) and centers ( $r^2 = 0.3622$ ) .



**Figure 4.** Species turnover between different habitat types and sites. A) NMDS plots based on 3 % OTUs, showing community similarity of all sampled sites. The plot shows community samples from barren lava, kīpuka centers, kīpuka edges, and continuous forest in Stainback and Kona Hema. The point sizes used to represent for kīpuka scales with kīpuka area. B) Community dissimilarity based on 3 % radius OTU beta diversity between site pairs within different types: lava, edge, center, Stainback, and Kona. C) NMDS plots based on zOTUs, showing community similarity of all sampled sites, designed as in panel A. D) Community dissimilarity based on zOTU beta diversity between site pairs, designed as in panel B.



**Figure 5.** Correlation of geographic distance between sample sites but within a habitat type, on a log scale, with community turnover, measured in terms of Bray Curtis dissimilarity for (A) 3 % OTUs and (B) zOTUs. The plots show dissimilarity between pairs of sites embedded in continuous forest in Stainback and in Kona Hema on the left, and between pairs of kīpuka sites (separated by lava) sampled from kīpuka centers and edges on the right.