Testing alternative hypotheses on the origin and speciation of Hawaiian katydids

Mohan Rakesh¹, Stephane Aris-Brosou¹,² and X. Xia¹,³*

Abstract

Background: Hawaiian Islands offer a unique and dynamic evolutionary theatre for studying origin and speciation as the islands themselves sequentially formed by erupting underwater volcanos, which would subsequently become dormant and extinct. Such dynamics have not been used to resolve the controversy surrounding the origin and speciation of Hawaiian katydids in the genus Banza, whose ancestor could be from either the Old-World genera Ruspolia and Euconocephalus, or the New World Neonoconocephalus. To address this question, we performed a chronophylogeographic analysis of Banza species together with close relatives from the Old and New Worlds.

Results: Based on extensive dated phylogeographic analyses of two mitochondrial genes (COX1 and CYTB), we show that our data are consistent with the interpretation that extant Banza species resulted from two colonization events, both by katydids from the Old World rather than from the New World. The first event was by an ancestral lineage of Euconocephalus about 6 million years ago (mya) after the formation of Nihoa about 7.3 mya, giving rise to B. nihoa. The second colonization event was by a sister lineage of Ruspolia dubia. The dating result suggests that this ancestral lineage first colonized an older island in the Hawaiian–Emperor seamount chain before the emergence of Hawaii Islands, but colonized Kauai after its emergence in 5.8 mya. This second colonization gave rise to the rest of the Banza species in two major lineages, one on the older northwestern islands, and the other on the newer southwestern islands.

Conclusion: Chronophylogeographic analyses with well-sampled taxa proved crucial for resolving phylogeographic controversies on the origin and evolution of species colonizing a new environment.

Keywords: Phylogeography, Biogeography, Geophylogeny, Speciation, DNA barcoding, Phylogenetics, Hawaii Islands
between the ancestors (typically on the mainland) and the descendants on the islands imposes differential selection and adaptation, leading to rapid phenotypic diversification on the island lineages in a short time [6], presumably aided by the small founding population that allowed the island lineage to escape a local fitness peak constrained by epistatic interactions. Fourth, geographic isolation among the islands and the associated rarity in inter-island gene flow lead to genetic isolation and speciation events that are relatively easy to detect and characterize by molecular phylogenetics and phylogeography [7, 8].

To demonstrate the power of such approaches, we here focus on the origin and speciation of the ten endemic Hawaiian katydids in the Genus Banza. This group of insects has become flightless with reduced wings. This limitation on their dispersal ability enhanced the genetic isolation and speciation process on the individual islands. In contrast, a winged grasshopper such as Schistocerca nitens was capable of dispersing from Hawaii to Nihoa by trade winds [9]. Both trade winds and extreme storms appear to have contributed to the observed biogeographic patterns in Hawaii Islands [10].

The pioneering study of Hawaiian katydids by Shapiro et al. [11] aimed to address four key questions associated with the origin and speciation of these flightless katydids. How many colonization events had occurred that resulted in subsequent origin and speciation of the katydids? Do the Banza species form a monophyletic taxon associated with a single colonization event? What is the ancestral lineage (or lineages) from which the Hawaiian katydids were derived? Are the ancestral lineages from the coastal region of eastern Asia or western coast of America? These questions are closely related and answering one of them will shed light on the others. However, the limitation of sampling in that study, as discussed by Shapiro et al. [11], did not allow these questions to be fully answered.

Three katydid genera, all featuring strong fliers, have been suggested Shapiro et al. [11] as potential ancestral lineages of the Banza species: Euconocephalus and Ruspolia in the Old World, and Neoconocephalus in the New World. Therefore, determining the ancestral lineage also provides information on the ancestral region of the Banza species. The study by Shapiro et al. [11] includes Neoconocephalus triops and two other North American species that diverged before the common ancestor of Neoconocephalus and Banza species, but no species from the Old World such as Euconocephalus or Ruspolia which is acoustically similar to Neoconocephalus and Banza species [12]. These two Old World genera were also missing in a recent phylogenetic study involving Neoconocephalus species [13]. Consequently, such studies cannot discriminate between the two alternative origin hypotheses, i.e., from Euconocephalus or Ruspolia lineages in the Old World or from Neoconocephalus in the New World.

Complete mitochondrial genomes are now available for Euconocephalus nasutus (the type species) and E. pallidus, as well as for Ruspolia dubia and R. lineosa. Their inclusion in the phylogenetic analysis is essential because insufficient taxon sampling may lead to misleading conclusions, as discussed in Shapiro et al. [11]. We contrast two extreme hypotheses in Fig. 1. The red lines depict a single colonization followed by successive island-hopping events that occurred as new islands emerged (Fig. 1A). This scenario is consistent with the phylogenetic results in Shapiro et al. [11], with the Banza species forming a monophyletic clade. The green lines represent a scenario with repeated colonization events from the mainland, but each colonization event was followed by wing loss before the emergence of the next island, so the flightless katydids could not colonize new islands that emerge through later volcanic activities (Fig. 1B). If mainland lineages X, Y and Z in Fig. 1B were never sampled, then all island species would superficially constitute a monophyletic taxon, with lineage W misidentified as the closest phylogenetic relative of all island species. Consequently, the scenario in Fig. 1B would be indistinguishable from that in Fig. 1A. For this reason, Shapiro et al. [11] cautioned a simple-minded interpretation of their phylogenetic tree which superficially suggests that Hawaiian katydids were derived from a single colonization event by an ancestor closely related to Neoconocephalus triops.

We performed phylogenetic analysis of the Banza species with the addition of species from Euconocephalus and Ruspolia, with extensive likelihood-based and Bayesian inference to improve on the previous phylogenetic analysis [11] without Euconocephalus and Ruspolia species. Our results suggest two successful colonization events that resulted in the extant Banza species. The first colonization event occurred about 6 mya by an ancestor of Euconocephalus, which gave rise to B. nihoa. The second colonization event occurred indirectly. An ancestor closely related to Ruspolia dubia colonized an island in the Hawaiian–Emperor seamount chain around 8.8 mya before the emergence of Hawaii Islands, and subsequently colonized Kauai and the younger islands after their sequential emergence. This gave rise to all other Banza species. These Euconocephalus and Ruspolia lineages inhabit eastern and southeastern Asia, including the coastal regions, so the ancestral lineage of Banza species is inferred to be from Asia instead of from America.
Results

Hawaiian katydids descended from Old World copiphorines in two colonization events

Our phylogenetic reconstructions with maximum likelihood (PhyML and RAxML) produced the same topology and nearly identical branch lengths (Fig. 2), which is expected because the same substitution model (GTR + Γ) has been used for both PhyML and RAxML. The PhyML tree is shown (Fig. 2). The topology from the two likelihood-based methods is also identical to that of the Bayesian tree from MrBayes (Fig. 3). The branch lengths of the Bayesian tree differ from those of the likelihood tree because of a slightly different substitution model (GTR + Γ + I) was used for MrBayes. The subtree support indicated by the posterior probabilities from the Bayesian inference (Fig. 3) appears higher than by the bootstrap support from the likelihood method (Fig. 2).

The trees in Figs. 2, 3 show that the ancestors of Hawaiian katydids are copiphorines in the Old World, related to Ruspolia and Euconocephalus. A previous study including only Neoconocephalus triops, but not Ruspolia and Euconocephalus, suggests that the ancestor of Hawaiian katydids is closely related to New World copiphorines [11, 15]. However, the original authors emphasized that the ancestor of Hawaiian katydids are equally likely to be from Ruspolia and Euconocephalus [11, 15]. Our results have now resolved this uncertainty: not only are Hawaiian katydids derived from Old World copiphorines, but there are also two independent colonization events leading to extant Hawaiian katydid lineages, both associated with strong bootstrap (Fig. 2) and posterior (Fig. 3) support.

The first colonization is by an ancestor (Node 1 in Figs. 2, 3) of the species in Euconocephalus giving rise to Banza nihoa (Figs. 2, 3), and the second by an ancestor (Node 2 in Figs. 2, 3) of R. dubia giving rise to the rest of Banza species (Figs. 2, 3). Thus, the genus Banza is paraphyletic in the sense that it does not include R. dubia and the two Euconocephalus species. The genus Ruspolia is polyphyletic because the two Ruspolia species are from separate lineages with their respective sister taxa. Banza nihoa is morphologically, acoustically, behaviorally and
ecologically different from the rest of the *Banza* species [11, 15], and was tentatively placed in a different genus [15]. Our phylogeny offers an evolutionary explanation for the differences recorded between *B. nihoa* and the rest of the *Banza* species, i.e., *B. nihoa* is a distinct lineage from the rest of *Banza* species in Hawaii Islands.

The phylogenetic relationship in Fig. 2 is consistent with other studies using 18S and 28S rDNA, cytochrome oxidase II, histone 3, tubulin alpha 1 and wingless genes [16]. Indeed, *Conocephalus* is the sister group of Copiphorinae which splits between *Neoconocephalus* and *Euconocephalus + Ruspolia*. The tree is also similar to that in Shapiro et al. [11] if we remove *Ruspolia* and *Euconocephalus* species. However, such removal would give a misleading impression that *Neoconocephalus* is the sister lineage of the *Banza* species in Hawaii Islands and that *Banza* is a monophyletic taxon. This exemplifies the importance of thorough taxon sampling. We should note that *Ruspolia* and *Euconocephalus* are closely related and still have species transferred from one genus to the other.

For example, *Euconocephalus indicus* is now considered as a synonym for *Ruspolia indica* [17].

**Fig. 2** Phylogenetic trees from PhyML analysis. Bootstrap support values are shown near internal nodes, with the two key support values (99.9 and 94.8) associated with the two colonization events highlighted in red. The RAxML tree has identical topology and nearly identical branch lengths, but with slightly lower bootstrap values. Four key internal nodes are numbered and referred to in the text as Nodes 1, 2, 3 and 4, respectively.
at Node 1, say 10 mya, before the emergence of any current Hawaiian Islands. Therefore, this ancestral island diverged from the mainland lineage 10 mya. Subsequently, this ancestral lineage colonized younger island as it emerged from the ocean. In this scenario, the age of the younger island should not be used to set the earliest time of Node 1 ancestor because its divergence from the mainland lineage occurred long before the emergence of the younger island. On the other hand, it is reasonable to assume that genetic diversification of katydid lineages among islands could not occur before the emergence of the islands. For example, descending lineages of Node 3 (Figs. 2, 3) evolved and diversified among Kauai and younger islands. We may limit the age of Node 3 as no earlier than the emergence of Kauai (~5.8 mya).

**Dating approach 1**

We dated the ancestors in two ways. The first assumes that all colonization events are independent and directly from the coastal regions of Asia. This limits Node 1 to be no earlier than the emergence of Nihoa (~7.3 mya) and Node 2 to be no earlier than the emergence of Kauai (~5.8 mya). With these restrictions, the first colonization event that gave rise to *Banza nihoa* was dated to about 6 million years ago (mya) (Fig. 4), after the emergence of Nihoa Island dated at 7.3 [2, 14]. The second colonization event that gave rise to the rest of *Banza* species was dated to 3.2 mya. The oldest lineage from this second colonization event was *B. unica* currently inhabiting Oahu Island that emerged about 3.9 mya [2, 14].

Because the specimens of *Banza* species have geographic coordinates, we constructed a geophylogeny of the two colonization events (Fig. 5). From East Asia, a sister lineage of the common ancestor of *Euconocephalus* colonized Nihoa Island, giving rise to *Banza nihoa* about 5.9 million years ago (Fig. 4). As Nihoa Island was dated to have emerged about 7.3 mya, while the first colonization event took place around 5.9 mya, the individuals of
this first colonization must have quickly become flightless before Kauai Island, which appeared about 5.8 mya (Fig. 5), became inhabitable. Consequently, B. nihoa was confined to Nihoa. Loss or gain of wings occur frequently in the stick insects in which fully winged, partially winged and wingless species originated multiple times independently [20]. We discuss later the benefit and cost of different wing morphs in isolated ocean islands.

The second colonization event was realized by a sister lineage of R. dubia ca. 3.2 mya, so the initial colonization could have occurred on Kauai emerged about 5.8 mya. However, only a young Banza lineage (B. kauaiensis) was found on Kauai. Thus, either the ancient Banza lineage from this second colonization have gone extinct on Kauai, or the colonizers first arrived on the next island that emerged 3.9 mya. The most ancient lineage from this second colonization event is B. unica found only on Oahu.

This second colonization event was then followed by island-hopping to colonize younger islands. The sister lineage of B. unica diverged into two lineages. The northwestern lineage is represented by B. kauaiensis, B. parvula, B. deplanata, and B. molokaensis colonizing the older islands (Kauai, Oahu and Molokai). The southeastern lineage consists of B. nitida, B. mauiensis, B. pilimauiensis, and B. brunnea and colonized the younger islands Maui and Hawaii (Fig. 5). The sequence divergence among the three B. nitida specimens are larger than that between B. kauaiensis and B. parvula or between B. brunnea and B. pilimauiensis (Figs. 2–3), prompting Shapiro et al. [11] to suggest the existence of cryptic species within B. nitida.

**Dating approach 2**

The dating approach above by using the age of Nihoa and Kauai as the maximum age of Node 1 and Node 2 ancestors may not be appropriate. The trees in Figs. 2, 3 indicate that Node 2 could actually be older than Node 1. It is possible that the Node 2 ancestor colonized an older island at time $T_2$ and then hopped onto Kauai at $T_2'$ after

---

**Fig. 4** Dated phylogenetic trees from MrBayes. The horizontal scales are in million years. Bars at the internal nodes show the 95% Highest Posterior Densities for date estimates. Numbers near internal nodes show mean dates. The red, green and blue arrowheads indicate the age of Nihoa, Kauai and Oahu, respectively.
its emergence. In this case, \( T_2 \) is earlier than the emergence of Kauai or even earlier than the emergence of Nihoa. For this reason, we should not limit the \( T_2 \) as no earlier than the emergence of Kauai. However, one may reasonably assume that genetic diversification of katydid lineages among islands could not occur before the emergence of the islands. For example, descending lineages of Node 3 (Figs. 2, 3) evolved and diversified among Kauai and younger islands. We may limit the age of Node 3 as no earlier than the emergence of Kauai (~ 5.8 mya).

This relaxation of age restriction on \( T_2 \) leads to new dating results in Fig. 6. While \( T_1 \) (Fig. 6) is still about 6 mya, \( T_2 \) is now 8.8 mya, earlier than Nihoa. This is consistent with the hypothesis that the Node 2 ancestor diverged from the mainland lineage about 8.8 mya and colonized an older island in the Hawaiian–Emperor seamount chain. This ancient island lineage subsequently colonized Kauai, Oahu and the younger islands as they emerged from the ocean.

One may note a trichotomy in Fig. 6 with three descending lineages (Node 1, Node 2 and \( R. \) lineosa). This trichotomy is not present in Fig. 4 where Node 2 is constrained by a calibration time. The reason for this trichotomy in Fig. 6 may be visualized from unrooted trees in Figs. 2, 3. Node 4 in Figs. 2, 3 has two descending lineages, Lineage 1 represented by \( R. \) lineosa and Lineage 2 represented by all other descendants from Node 4. It is easy to see that the distance from Node 4 to \( R. \) lineosa (\( D_1 \)) is smaller than the average distance from Node 4 to the 24 descendants of Lineage 2 (\( D_2 \)). Fitting a molecular clock would tend to force \( D_1 = D_2 \), which favors a shift of Lineage 2 towards the root, and eventually merging the ancestor of Lineage 2 with Node 4 leading to a trichotomy.

The dating results in Fig. 6 leads to a different geophylogeny in Fig. 7. By 4.4 mya, this island lineage colonizing Kauai had already diverged into two lineages (referred hereafter as Lineage 1 and Lineage 2). Lineage 1 initially diverged in Kauai and Oahu. Around 2.1 mya when Molokai was formed, Lineage 1 colonized Molokai. We designate the original Lineage 1 on Kauai and Oahu as Lineage 1.1 and those colonized Molokai as Lineage 1.2. Lineage 1.1 speciated into \( B. \) kauaiensis on Kauai Island and \( B. \) parvula on Oahu Island. Lineage 1.2 speciated into \( B. \) molokaiensis on Molokai and \( B. \) deplanata on Lanai. Note that the divergence time of \( Banzia \) species could be older than the age of the island they inhabit because the katydids arriving at the island

![Fig. 5 Geophylogeny of Hawaiian katydids and their relatives, generated from PGT [21]. Shaded areas in East Asia inset represents the distribution of \( Ruspolia \) and \( Euconocephalus \) on the coastal and island regions. Vertical branches in the geophylogeny of Hawaiian katydids represent branch length. Divergence time in dated internal node is shown in million years (my). The geological age of each island is shown next to the islands. Species names associated with the letters at the tips of the geophylogeny are listed in the lower-left of the figure.](image-url)
Lineage 2 initially colonized Oahu and diverged into two lineages, Lineage 2.1 and Lineage 2.2. Lineage 2.1 becomes the extant B. unica remaining in Oahu. Lineage 2.2 colonized the newest southeastern islands, i.e., Maui and the Big Island as they emerge from the Pacific Ocean, and speciated into B. brunnea, B. mauiensis and B. pilimauiensis on the island of Maui, and B. nitida on the Big Island. As B. nitida has already become flightless, the new island that is forming underwater to the southeastern side of the Big Island most likely would need new colonization by winged mainland populations from the Old World or the New World.

Discussion
Long-distance migration, colonization, speciation and extinction
While it remains a matter of debate how the ancestors of Hawaiian katydids crossed the vast span of the Pacific to arrive at the Hawaiian Islands, such long-range migration is possible for two reasons. First, the earth has undergone cold and warm periods. During cold glaciation period, much ocean water was locked on land in the form of thick icesheets, with sea level much lower than it is today. For example, the ice sheet in Antarctica expanded to its maximum around 5.6 mya, contributing to the decrease in sea level and the complete drying-up of the Mediterranean Sea [5]. This corresponds roughly to the inferred colonization time of the Nihoa Island. The thick icesheets on the land and the consequent reduction in sea level imply that many islands under water in the Pacific today were above water level during glaciation period, eliminating the necessity of colonizers flying for thousands of kilometers in a single flight. Second, Ruspolia species, e.g., R. differens, are strong flyers, comparable to their relatives such as locusts and grasshoppers. For instance, in 1988, swarms of desert locust (Schistocerca gregaria) flew across the Atlantic Ocean covering about 5,000 km from West Africa to the Caribbean Islands in ten days.
prompting the proposal that the New World *Schistocerca* species were derived from ancestral African lineages [22, 23]. This contrasts with the alternative hypothesis that the common ancestor of *Schistocerca* lived before the separation of Africa and South America and that the Old World *Schistocerca* and New World *Schistocerca* lineages are sister lineages resulting from the separation of the two continents. Only molecular phylogenetics with sufficient taxon sampling and accurate dating can fully resolve such alternative hypotheses. The point we wish to make here, however, is that it is not inconceivable for the Asian ancestors of katydids to cross the ocean and colonize Hawaiian Islands.

The example of transatlantic locust migration by desert locusts above does highlight the uncertainty underlying phylogeographic studies. If such a species becomes established in America and, after millions of years, loses its ability of transatlantic flight, the phylogeographic pattern may mislead researchers to conclude that the species was present before the separation between Africa and America but have evolved very slowly ever since. Similarly, one may hypothesize that extreme storms blowing westward contribute significantly to biogeography in the Pacific islands [10] and gain undeserved support of the hypothesis. For example, if we remove *Euconocephalus* and *Ruspolia* species from Figs. 2, 3, 4, 5, 6, 7, then the pattern would be supportive of this hypothesis because *Banza* species appear to have descended from the New World *Neoconocephalus*. For this reason, we should follow Shapiro et al. [11] by cautioning readers of possible pitfalls in phylogeographic interpretations.

Katydids have colonized Hawaiian Islands many times, but only those with extant lineages are observable. *Conocephaloides hawaiiensis* [24], with fully developed wings and extraordinarily long femurs, may have gone extinct as it had not been found with extensive field sampling [15]. Only a color drawing of *C. hawaiiensis* remains [24], together with a brief description on how it can be distinguished morphologically from *Conocephalus* and *Brachymetopa* species. *Brachymetopa* is a synonym of *Banza*, and *Conocephaloides* species have been assigned to *Euconocephalus, Ruspolia* and *Neoconocephalus* [17]. *Euconocephalus remotus* was on IUCN’s Red List of Threatened Species in 1996 and likely has gone extinct [11, 15, 25]. This highlights the urgency of sampling species endemic in Hawaii Islands.

The population decline and extinction of certain Hawaiian katydids may be caused by inadvertently
introduced species, such as grasshoppers, that could severely damage vegetation in a large scale [9]. In addition to inadvertently introduced species, many “beneficial” species have been introduced into Hawaiian Islands, starting with the ladybeetle *Novius cardinalis* in 1890 [26, 27]. Unfortunately, thousands of species introduced into Hawaiian Islands were not recorded [27]. A large-scale barcoding project would help identify these introduced species as they should exhibit little genetic differentiation from their respective source populations. It would indeed be a shame for ecologists and evolutionary biologists not to reap scientific understanding from such a large-scale experiment on introduced species.

**Evolution of wing-loss**

Given the success of insects, there must be an evolutionary advantage of flight [28]. Such advantage includes the efficiency in finding and locating new food, nesting and mating resources. Limited evidence suggests that such advantages may be greater in males than in females. Whenever there is sexual dimorphism in wings in stick insects, it is always the male that has more wings [20]. However, in spite of the advantage of flight, the loss of flight in insects evolved multiple times in the Hawaiian Islands in 10 of the 11 orders of insects, often with the alate and flightless sister species living side by side [6].

There are two hypotheses invoking a fitness advantage for the loss of flight. Darwin’s original proposal [29] is that the chance of being blown into the sea is greater than the benefit of finding better resources. If a population lives in a small, windy and isolated island, then the cost is obviously great and the benefit little. This would predict an inverse relationship between the propensity of wing loss and the size of an island, and he cited the more apterous forms in the small islet Dezerta Grande than the larger Madeira to support his argument. The same association has also been observed in Hawaii Islands. The introduced and fully winged *Schistocerca nitens nitens* took flight more readily in the large Hawaiian Islands than in smaller ones [15]. This relationship between windy and spatially confined environment and insect wing loss was extensively documented subsequently [28, 30–33]. This hypothesis has been extended to include the energetic cost of maintaining wings in a windy environment [34].

The second hypothesis, implicitly alluded to by Roff [28] and Whiting et al. [20], invokes the benefit of occupying vacant niches. Mainland habitats invariably features many non-flying terrestrial and subterranean arthropods. These non-flying arthropods are few in remote islands because of their limited dispersal capability. This implies that the ecological niches typically occupied by such non-flying arthropods are vacant in remote islands. Consequently, there is potential benefit for some insects to lose their wings to fill in such niches. After all, almost all insects, including the strongest fliers, spend much of their life without using wings. This second hypothesis is applicable not only to islands, but also to high-rising mountain peaks with isolated populations. When such an isolated population of non-flying arthropods go extinct, their ecological niches are vacated and not readily filled by other non-flying arthropods living in other isolated peaks. Thus, we expect insects to have an increased propensity to lose their flight and become ground-dwelling in habitats where non-flying arthropods are rare. The two hypotheses outlined above are not mutually exclusive. Rather, they highlight different aspects of potential fitness gain. Hawaiian Islands would serve as an ideal place for evaluating the relative importance of these two hypotheses.

**Can the island population from Hawaii back-colonize the mainland?**

One of the reviewers suggested the possibility of *R. dubia* originated as a consequence of back-colonization of Asian mainland. That is, after establishing an island population in Nihoa, some descendants of the original colonizers flew back to the Asian mainland and evolved into *R. dubia*. This suggestion is likely prompted by the observation that species in *Ruspolia* and *Euconocephalus* are often strong fliers. If they could fly from Asian mainland to Hawaii, then it is theoretically possible to make a return trip, especially because extreme storms tend to blow westward [10].

Three lines of reasoning would go against such a back-colonization hypothesis. First, even fully winged insects in Hawaii Islands appear to refrain from taking flight, with the consequence that few insect species are found in more than one island [15]. This is consistent with the hypothesized cost of alate insect forms being blown by wind into open water and drowned. The recently introduced grasshopper (*Schistocerca nitens nitens*) is fully winged and can still fly in larger islands but rarely do so in small islands such as in Nihoa [15]. Second, long-distance migration in insects is typically preceded by a very large population with the consequent swarming behavior. Insect populations on remote islands of limited sizes typically do not reach a population size large enough for swarming, so back colonization is unlikely. Third, in contrast to Hawaiian Islands which emerged as volcanic islands with hardly any biodiversity, the mainland habitat is typically rich in a variety of insect species. Therefore, even if a few exhausted katydids from Hawaii Islands made their way to the mainland, they would be unlikely to win against established and well-adapted mainland competitors.
The importance of having large-scale phylogeographic data

Phylogeographic studies require dense sampling because the fundamental question addressed by phylogeography is why a species X is present in Area A but not in Area B. The distribution pattern of species X could have four causes. First, X has limited dispersal capability and has never had a chance to arrive in Area B. Second, X did disperse to Area B but could not survive and reproduce in Area B. Third, X has arrived in Area B, survived and reproduced, but has evolved into species Y. Fourth, X has arrived in Area B, survived and reproduced, but was not encountered and sampled by the researcher.

This conceptual framework is relevant in interpreting the distribution pattern of Banza species after the second colonization event (Figs. 2, 3, 4, 5, 6, 7). These colonizers must have first landed in Kauai and then Oahu because the younger islands have not emerged yet. One therefore would expect the highest genetic diversity and the most ancient Banza lineages to be found in either Kauai or Oahu. However, the two B. kauaiensis specimens found in Kauai are neither ancient nor genetically diverse. The extensive sample collection [15] decreased the plausibility of the hypothesis that ancient Banza lineages are still present in Kauai but not sampled. One may therefore conclude that the ancient Banza lineages on Kauai had gone extinct and the island was re-colonized by recently derived B. kauaiensis. One cannot reach such a conclusion without the extensive sample collection [15].

Conclusions

We have shown here how phylogeographic analysis could help resolve alternative hypotheses on the origin and evolution of species colonizing a new environment and how it is crucial to have sufficient taxon sampling. Our results revealed the origin of Hawaiian Banza katydids by two colonization events, both by katydids from the Old World rather than from the New World. The first colonization was by an ancestral lineage of Euconocephalus about 6 mya after the formation of Nihoa about 7.3 mya, giving rise to B. nihoa. The second colonization was by a relative of Ruspolia dubia giving rise to the rest of Banza species in two major lineages, one lineage inhabiting and diverging on the older northwestern islands and the other on the newer southwestern islands. The phylogenetic pattern revealed by our analysis suggests the need of an extensive revision of the taxonomic status of species in Euconocephalus, Ruspolia and Banza.

Methods

Sequence retrieval

The mitochondrial COX1 and CYTB genes for the 23 specimens including ten species of Hawaiian katydids (genus Banza) and three outgroup species were downloaded from NCBI (see Additional file 2: Table S1 for accession numbers). Sequences from three sets of potential direct ancestors belonging to the family Tetttigonioidea, subfamily Conocephalinae and tribe Copiphorini were also retrieved: (1) Ruspolia dubia and R. lineosa; (2) six Euconocephalus indica individuals; and (3) one belonging to Neoconocephalus. The first two species are distributed in eastern and southeastern Asia, including the coastal regions, while Neoconocephalus species are distributed in America, including western coastal regions [35].

As outgroups, we use three katydid species belonging to the same subfamily but a different tribe (Conocephalini): Conanalus pieli, Conocephalus maculatus, and Conocephalus melanus. Also included are two phylogenetically more distant katydid species, Ducetia japonica of the subfamily Phaneropterinae, and Pseudokuzicus pieli of the subfamily Meconematinae.

Sequence alignment, dating, and phylogeographic analyses

Complete mitochondrial genomes (mtDNAs) were parsed with DAMBE [36, 37]. The COX1 and CYTB sequences were aligned using MAFFT [38] with the most accurate LINSI option (‘--localpair’ and ‘--maxiterate=1000’). Consistent with the mostly non-recombining nature of the mtDNA, the two mitochondrial genes have a concordant phylogenetic signal [11], so that initial phylogenetic analyses were done on the concatenated sequences.

For phylogenetic reconstruction with PhyML v3.3 [39] and RAxML [40], we used jModelTest v.2.1.10 to select the best substitution model [41] based on an estimated BIONJ tree [42] under each model. Table S2 shows the log-likelihood for different models based on the BIONJ tree, with four discrete rate categories for approximating a continuous gamma distribution [43]. For PhyML, the tree improvement option (‘-s’) was set to ‘BEST’ (best of NNI and SPR search). The ‘-o’ option was set to ‘thr’ which optimizes the topology, the branch lengths and rate parameters. RAxML performs 1000 rapid bootstrap inferences and a thorough ML search. Geophylogenies (i.e., phylogenetic trees mapped onto a geographic map to highlight the spatial and temporal partition of biodiversity) were generated from PGT software [21].

For Bayesian phylogenetic reconstruction with MrBayes v3.2.7 [44], the GTR substitution model was used with gamma-distributed rate variation across sites, and with or without estimating a proportion of invariant sites. For all Bayesian analyses, the run length was increased until the standard deviation of split frequencies is less than 0.01. The sump command shows PSRF (potential scale reduction factor) varying
from 1.000 to 1.004, and average ESS (effective sample size) varying from 530 to 1054, for rate and frequency parameters. The resulting cladogram with the posterior probabilities and the phylogram with mean branch lengths were visualized in FigTree [45].

In order to date these phylogeographic patterns, relaxed molecular clock analyses were performed. We used MrBayes v3.2.7 [44] with the independent gamma rates (IGR) model. The clock was calibrated based off the timing of the volcanic emergence of each island, and hence did not employ minimum ages as is usually the case [18], but maximum ages by means of uniform distributions. Two dating approaches were used, differing in calibration priors. The first restricts the age of Node 1 (Figs. 2, 3) to no earlier than the emergence of Nihoa (~7.3 mya) and the age of Node 2 (Figs. 2, 3) no earlier than the emergence of Kauai (~5.8 mya). The second removed the restriction on Node 2 because the trees in Figs. 2, 3 suggest that the second colonization may involve the colonization of an island older than the current Hawaii Islands, with the ancestral island lineage colonizing Kauai later. Because the descendants of Node 3 (Figs. 2, 3) have been observed only in Kauai and younger islands, it is assumed that their genetic diversification among the island species cannot be earlier than the emergence of Kauai (~5.8 mya). Figures 4, 5 are from the first dating approach and Figs. 6, 7 from the second dating approach. For all dating analysis, the GTR + \Gamma substitution model was used, without estimating the proportion of invariant sites.

**Acknowledgements**

We thank J. Starr and members in Xia Lab for discussion and comments. Three anonymous reviewers provided excellent comments and suggestions that substantially improved the quality of the manuscript.

**Author contributions**

XX conceived the project. XX, SAB and MR performed data analysis. XX and SAB drafted and revised the manuscript. All authors read and approved the final manuscript.

**Funding**

This study was funded by the Discovery Grant from Natural Science and Engineering Research Council (S.A.-B., X.X.) of Canada for basic research (RGPIN/2018-03878). The funder played no role in this work.

**Availability of data and materials**

All data needed to duplicated results in the paper were retrieved from NCBI (https://ncbi.nlm.nih.gov/) and BOLD (https://www.boldsystems.org/). Both are public databases open to everyone. All accession numbers have been provided in Additional file 2: Table S1. Aligned sequences are included as Additional file 1.

**Declarations**

**Ethics approval and consent to participate**

Inapplicable.

**Consent for publication**

Inapplicable.

**Competing interests**

The authors declare that they have no competing interests.

**Author details**

1. Department of Biology, University of Ottawa, 30 Marie Curie, Station A, P.O. Box 450, Ottawa, ON K1N 6N5, Canada. 2. Department of Mathematics and Statistics, University of Ottawa, Ottawa, ON K1N 6N5, Canada. 3. Ottawa Institute of Systems Biology, Ottawa, ON K1H 8M5, Canada.

Received: 9 July 2021 Accepted: 13 June 2022

**Published online: 22 June 2022**

**References**

1. Neall VE, Trewick SA. The age and origin of the Pacific islands: a geological overview. Philos Trans R Soc Lond B. 2008;363:3293–308.
2. Clague DA. Growth and subsidence of the Hawaiian-Emperor volcanic chain. In: Keast A, Miller SE, editors. The origin and evolution of Pacific Island biotas, New Guinea to Eastern Polynesia: patterns and processes. Amsterdam: SPB Academic Publishers; 1996. p. 35–50.
3. Wilson JT. A possible origin of the Hawaiian Islands. Can J Phys. 1963;41(6):863–70.
4. Howarth FG, Mull WP. Hawaiian insects and their kin. Honolulu, HI: University of Hawaii Press, 1992.
5. Ohneseis C, Florindo F, Stocchi P, Roberts AP, DeConto RM, Pollard D. Antarctic glacio-eustatic contributions to late Miocene Mediterranean desiccation and reflooding. Nat Commun. 2015;6(1):8765.
6. Howarth FG. Hawaiian terrestrial arthropods: an overview. Bishop Mus Occas Papers. 1990:30:4–26.
7. Avise JC. Phylogeography: the history and formation of species. Cambridge, MA: Harvard Univ. Press; 2000.
8. Brooks DR, McLennan DA. Phylogeny, ecology and behavior: a research program in comparative biology. Chicago: University of Chicago Press; 1991.
9. Latchininsky AV. Grasshopper outbreak challenges conservation status of a small Hawaiian Island. J Insect Conserv. 2008;12(3):343–57.
10. Gillespie RG, Baldwin BG, Waters JM, Fraser CI, Nikula R, Roderick GK. Long-distance dispersal: a framework for hypothesis testing. Trends Ecol Evol. 2012;27(1):47–56.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s12862-022-02037-2.

Additional file 1. Aligned sequences, with COX1 and CybT separately aligned and then concatenated.

Additional file 2: Table S1. Accession numbers of the sequences used in this study. Species isolates / vouchers, where applicable, are shown between parentheses. Accession numbers are shown for the COX1 and cybt genes. Two accession numbers are for partial COX1 and CYTb sequences, respectively; complete mitochondrial sequences with full-length COX1 and CYTb sequences were indicated by a single accession number. Table S2. Model selection results. Twelve models were tested, with \( \Lambda \) components based off the four-category discretization. Number of parameters (\( p \)), Akaike Information Criterion (AIC), and distance from best (minimal) AIC score (\( \Delta AIC \)) are shown.
11. Shapiro LH, Strazanac JS, Roderick GK. Molecular phylogeny of Banza (Orthoptera: Tettigoniidae), the endemic katydids of the Hawaiian Archipelago. Mol Phylogenet Evol. 2006;41(1):53–63.

12. Greenfield MD. Evolution of acoustic communication in the genus Neocconocephalus: discontinuous songs, synchrony, and interspecific interactions. In: Bailey WJ, Rentz DCF, editors. The Tettigoniidae: biology, systematics and evolution. Berlin: Springer; 1990. p. 171–97.

13. Snyder RL, Frederick-Hudson KH, Schul J. Molecular phylogenetics of the genus Neocconocephalus (orthoptera, tettigoniidae) and the evolution of temperate life histories. PLoS ONE. 2009;4(9): e7203.

14. Clague DA, Sherrard DR. Characteristics of Hawaiian Volcanoes. In:Poland MP, Takahashi TJ, Landowski CM, editors. US geological survey professional paper 1801, 2014, pp 97–146.

15. Strazanac JS. Hawaiian endemic Copiphorini: systematics and acoustics (Orthoptera: Tettigoniidae: Conocephalinae). Honolulu: University of Hawaii at Manoa; 1996.

16. Almgren JD, Song H, Whiting MF. A century of paraphyly: a molecular phylogeny of katydids (Orthoptera: Tettigoniidae) supports multiple origins of leaf-like wings. Mol Phylogenet Evol. 2013;69(3):1120–34.

17. Eades DC, Otte D, Cigliano MM, Braun H. OrthopteraSF: Orthoptera Specialist Group: Euconocephalus remotus. IUCN Red List of Threatened Species 2020, 139(41):(Source: Biodiversity Heritage Library).

18. Byrne L, Chapleau F, Ars-Brosois S. How the Central American Seaway and an Ancient Northern Passage Affected Flatfish Diversification. Mol Biol Evol. 2018;35(8):1982–9.

19. Garland GD. John Tuzo Wilson, 24 October 1908–15 April 1993. Biogr. Mems Fellows R Soc. 1995;41:53–52.

20. Whiting MF, Bradler S, Maxwell T. Loss and recovery of wings in stick insects. Nature. 2003;421(6920):264–7.

21. Xie X. PGT: Visualizing temporal and spatial biogeographic patterns. Glob Ecol Biogeogr. 2019;28:1195–9.

22. Kevan DKM. Transatlantic travelers. Antenna. 1989;13:12–5.

23. Ritchie M, Pedgley DE. Desert locusts cross the Atlantic. Antenna. 1989;13:10–2.

24. Kane H. Orthoptera FAM. LOCUSTIDAE SUMFAM. COPIPHORINAE. Genera Insectorum 1912, 139(41):(Source: Biodiversity Heritage Library).

25. Orthopteroid Specialist Group: Euconocephalus remotus. IUCN Red List of Threatened Species 2019-2020(287):20202121.

26. Darwin C. To J. D. Hooker, 7 March [1855]. In: Darwin Correspondence Project. University of Cambridge; 1855.

27. Swezey OH. Records of introduction of beneficial insects into the Hawaiian Islands. Hawaii Plant Rec. 1925;29(4):369–76.

28. Roff DA. The evolution of flightlessness in insects. Ecol Monogr. 1990;60:389–421.

29. Darwin C. To J. D. Hooker, 7 March [1855]. In: Darwin Correspondence Project. University of Cambridge; 1855.

30. Harrison RG. Dispersal polymorphisms in insects. Annu Rev Ecol Syst. 1980;11:95–118.

31. Roff DA. Habitat persistence and the evolution of wing dimorphism in insects. Am Nat. 1994;144:772–98.

32. Mc Culloch GA, Foster BJ, Ingram T, Waters JM. Insect wing loss is tightly linked to the timeline: evidence from a diverse stonefly assemblage. Ecography. 2019;42:811–3.

33. Hodkinson ID. Terrestrial insects along elevation gradients: species and community responses to altitude. Biol Rev Camb Philos Soc. 2005;80:489–513.

34. Lehly RI, Chown SL. Wind plays a major but not exclusive role in the prevalence of insect flight loss on remote islands. Proc R Soc B: Biol Sci. 1940;2020(287):20202121.

35. Cigliano MM, Braun H, Eades DC, Otte D. Orthoptera species file. Version 5.0.5.0. In: 2021.

36. Xia X. DAMBE6: a comprehensive software package for data analysis in molecular biology and evolution. Mol Biol Evol. 2013;30:1720–8.

37. Xia X. DAMBE6: new tools for microbial genomics, phylogenetics, and molecular evolution. J Hered. 2017;108(4):431–7.

38. Katoh K, Aisimotos G, Toh H. Multiple alignment of DNA sequences with MAFFT. Methods Mol Biol. 2009;537:39–64.