



Diversity and clade ages of West Indian hummingbirds and the largest plant clades dependent on them: a 5–9 Myr young mutualistic system

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Received 23 September 2014; revised 10 November 2014; accepted for publication 12 November 2014

We analysed the geographical origins and divergence times of the West Indian hummingbirds, using a large clock-dated phylogeny that included 14 of the 15 West Indian species and statistical biogeographical reconstruction. We also compiled a list of 101 West Indian plant species with hummingbird-adapted flowers (90 of them endemic) and dated the most species-rich genera or tribes, with together 41 hummingbird-dependent species, namely *Cestrum* (seven spp.), *Charianthus* (six spp.), Gesnerieae (75 species, c. 14 of them hummingbird-pollinated), *Passiflora* (ten species, one return to bat-pollination) and *Poitea* (five spp.), to relate their ages to those of the bird species. Results imply that hummingbirds colonized the West Indies at least five times, from 6.6 Mya onwards, coming from South and Central America, and that there are five pairs of sister species that originated within the region. The oldest of the dated plant groups diversified 9.1, 8.5, and 5.4 Mya, simultaneous with or slightly before the extant West Indian bird radiations. The time frame of the coevolved bird/flower mutualisms obtained here resembles that recently inferred for North America, namely 5–9 Mya. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2015, **114**, 848–859.

ADDITIONAL KEYWORDS: Caribbean – coevolution – endemism – radiations – time trees.

INTRODUCTION

The West Indies consist of the Greater Antilles, which are continental fragments, the Lesser Antilles, which are mainly of volcanic origin, and the Bahamas, which developed from coral reefs since the Pliocene (Ricklefs & Bermingham, 2008). Most of the Greater Antilles has remained above sea level at least since the mid-Eocene, while the Lesser Antilles has been continuously reshaped by active volcanism since the Miocene (Iturralde-Vinent & MacPhee, 1996). Because of this complex geology, with large and small

islands of many different ages, the West Indian Archipelago has served as a natural laboratory for testing species formation on islands, adaptive radiation and colonization/extinction dynamics (Lack, 1973; Wilson, 1985; Losos & Schluter, 2000; Ricklefs & Bermingham, 2008; Burbrink *et al.*, 2012). Early middle Eocene rhinocerotoid land mammal fossils suggest the possibility of overland dispersal or stepping stone dispersal from Central America (Hedges, 2001; Dávalos, 2004), but most colonization events must have involved the crossing of large ocean expanses. The species-poor bird fauna of the West Indies, however, shows that sustained colonization of new islands, even for strong fliers, is a substantial challenge. Compared with other archipelagos, the

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West Indies has few bird radiations (Ricklefs & Bermingham, 2008), although exceptions exist in hummingbirds (Lack, 1973; Lance *et al.*, 2009; McGuire *et al.*, 2014), the focus of the present study.

The West Indies have 16 species of hummingbirds, 15 of them endemic (Schuchmann, 1999), with the highest number of sympatric species, five, found on Puerto Rico. Dominica, Martinique and Jamaica each have four species, some of them shared; Cuba has two and Jamaica has three endemics that clearly must have originated on these islands (Lack, 1973; Schuchmann, 1980; Lance *et al.*, 2009). For their nectar, West Indian hummingbirds depend on flowers from many plant species (Lack, 1976). Consistent bird pollination exerts selection pressure on flowers that over time will develop a typical syndrome of traits associated with birds as pollen vectors. These traits are (i) scentless brightly coloured, often red flowers (Scogin, 1983) with (ii) elongate corolla tubes, (iii) large amounts of sucrose-rich nectar, (iv) usually no landing platform, (v) diurnal opening, and (vi) stigmas and stamens placed such that foraging hummingbirds effectively cause cross-pollination (Stiles, 1981; Stiles & Freeman, 1993; Rosas-Guerrero *et al.*, 2014). Some of the best-studied co-evolved hummingbird/plant mutualisms in the West Indies involve *Heliconia bihai* and *H. caribaea* and male and female *Eulampis jugularis* on Dominica and St Lucia (Temeles & Kress, 2003; Temeles, Goldman & Kudla, 2005), and *H. bihai* and other species of hummingbirds on Grenada and Trinidad, islands outside the range of *E. jugularis* (Snow & Snow, 1972; Dalsgaard *et al.*, 2009, 2012).

The present study addresses the geographical and evolutionary build-up of the West Indian hummingbird fauna and species-rich local radiations of plants with flowers adapted to bird pollination. We also compare the ages of the West Indian hummingbirds and bird-adapted plant radiations (i.e. species with flowers showing the result of consistent selection from birds) with the oldest hummingbirds and bird-adapted plant clades in North America. In North America, hummingbird/plant interactions have evolved gradually over the past 7–5 Myr (McGuire *et al.*, 2014). The oldest hummingbird-adapted group in North America may be *Lonicera* (Caprifoliaceae), with a four-species bird-adapted clade that has a stem age of 9.2 Mya and a crown age of 7.0 Mya (Smith & Donoghue, 2010). The North American bee hummingbirds diverged from Central and South American relatives about 6.8 Mya and radiated 5.6 Mya (McGuire *et al.*, 2014). By contrast, some Andean hummingbird/plant co-evolutionary systems are roughly twice as old, with interacting bird species and plant groups dating back about

11 Myr (Abrahamczyk, Souto-Vilarós & Renner, 2014).

A clock-dated phylogenetic tree for the hummingbirds recently clarified that the West Indian species resulted from at least five colonization events, apparently not earlier than 5 Mya (McGuire *et al.*, 2014). Building on these results, we here infer the geographical origin of 14 of the 15 endemic West Indian hummingbirds, using statistical biogeographical reconstruction (not done by McGuire *et al.*, who focused on hummingbird diversification rates not biogeography), and we then address the question of the overall time frame over which hummingbird-adapted plant groups have co-evolved with hummingbirds in the West Indies. To select the largest such plant groups, we compiled a list of all species with hummingbird-adapted flowers (Supporting Information, Table S1), and from the 90 endemic such species, selected those genera or tribes with the most bird-adapted species.

We expected the oldest hummingbird sister pairs in the West Indies and the oldest bird-dependent plant groups that have radiated on the West Indies to have similar ages, although extinction and re-colonization, asymmetric mutual dependencies and insufficient knowledge of the bird-adapted West Indian flora may make it difficult to detect such temporal matching.

MATERIAL AND METHODS

SEQUENCE DATA, TREE INFERENCE AND CLOCK CALIBRATIONS FOR THE HUMMINGBIRDS

We used 29 000 dated post-burn-in trees from a Markov chain Monte Carlo (MCMC) produced in BEAST v.1.7 (Drummond & Rambaut, 2007; Drummond *et al.*, 2012) by McGuire *et al.* (2014) from their 284-species alignment to construct a maximum clade credibility tree using TreeAnnotator v.1.7.5 (part of the BEAST package). McGuire *et al.* (2014) calibrated their trees with locus-specific rates of 0.00035–0.058 substitutions per site per Myr estimated for these loci in Hawaiian honeycreepers (Lerner *et al.*, 2011). These rates bracket the substitution rate of 0.0112 substitutions per site per Myr calculated for hummingbird mitochondrial *Cyt-b* by Chaves, Weir & Smith (2011).

To test the results obtained with the rate calibration, we fossil-calibrated a slightly smaller sequence alignment of 221 hummingbird and five outgroup species and 4022 nucleotides. For this alignment we newly sequenced two nuclear [intron 7 in the *beta fibrinogen* (BFib) gene and intron 5 in the *adenylate kinase* (AK1) gene, 2045 bp] and two mitochondrial markers (*NADH dehydrogenase* subunits 2 and 4;

ND2 and ND4, 1977 bp) of *Trochilus polytmus*, *T. scitulus* and two accessions of *Cyanophaea bicolor*, one from Jamaica and one from Martinique. DNA extraction and amplification followed McGuire *et al.* (2014). The origin of bird tissues and GenBank accession numbers are provided in Supporting Information Table S2. Calibration came from a stem group fossil from the oil shale of Messel in southern Germany that provide a minimum age for the divergence between hummingbirds and swifts (Mayr, 2009). Argon dating of igneous rocks underlying the Messel pit indicates a Lower Lutetian age of approximately 47.5 Myr (Schulz *et al.*, 2002), and this age has been accepted for these fossils. We used a gamma distribution with an offset at 47.5 Mya, a shape parameter of 2.1 and a median of 51.37, allowing 95% of the ages to fall between 48.1 and 60.0 Mya, and 2.25% to be older than 60.0 Mya. This permitted the stem age to go back to 60.0 Mya, encompassing the stem age of 58.5 Mya obtained by Bleiweiss (1998).

We ran strict and relaxed clock models, and then chose the model for which the ucl.d.stdev value in Tracer v.1.6 (Rambaut & Drummond, 2009) was ≥ 0.5 , which for our data was the strict clock. All runs employed a Yule tree prior as recommended for species-level analyses (BEAST manual). Modeltest (<http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html>) gave the GTR + G substitution model, followed by the HKY + G model, as best fitting the mitochondrial data (226 \times 1977 aligned nucleotides). The best-fitting model for the nuclear matrix, which comprised 2045 aligned nucleotides, was the K2P + G model. For the combined matrix we chose a substitution model of intermediate complexity, namely the HKY + G model. MCMCs were run for 20 million generations, sampling every 10 000th generation. Effective sample sizes for the evolutionary parameters were all above 300. Of the posterior 2000 trees, we dropped the first 20% as burn-in and then checked convergence, using Tracer. Maximum clade credibility trees were edited in FigTree v.1.4.0 (Rambaut, 2009), such that 95% confidence intervals are shown only for nodes with a posterior probability of $\geq 98\%$.

SEQUENCE DATA, TREE INFERENCE AND CLOCK CALIBRATIONS FOR THE PLANT LINEAGES

We compiled a list (Supporting Information, Table S1) of plant species with flowers showing the following traits: (i) brightly coloured, often red flowers with (ii) long corolla tubes, (iii) large amounts of sucrose-rich nectar, (iv) usually no landing platform, (v) diurnal flower opening, and (vi) stigmas and stamens placed such that foraging hummingbirds effectively cause

cross-pollination. We also searched the literature to find plant species pollinated by hummingbirds but not showing these traits. Plant species occasionally visited by hummingbirds but mainly pollinated by insects were not included. The species, along with references on their pollination mode and status as endemic or not, are shown in Supporting Information, Table S1. We focused on clock-dating the most species-rich West Indian bird-pollinated groups (*Cestrum*, *Charianthus*, *Gesneriaceae*, *Passiflora*, *Poitea*; cf. Supporting Information, Table S1) as well as a sample of phylogenetically isolated bird-pollinated species, trying to span what might be some of the oldest and youngest bird-adapted plants in the region.

Divergence times were obtained from relevant published phylogenies, newly clock-dated matrices from TreeBASE, slightly enlarged with GenBank sequences (see accession numbers in Supporting Information, Table S3), or from colleagues (see Acknowledgements). Sequences were aligned with Mesquite version 2.75. We employed relaxed clock models because Tracer indicated ucl.d.stdev values ≥ 0.5 for all phylogenies. In all BEAST runs, we used the Yule tree prior as recommended for species-level phylogenies and the GTR + G substitution model with four gamma rate categories. We ran MCMCs for 20 or 40 million generations, sampling every 10 000th generation. The first 20% of the posterior 2000 trees were discarded as burn-in, and the remaining data were then checked for convergence in Tracer. Trees were edited in FigTree as done for the bird dating.

Absolute times (Mya) were obtained for eight clades as follows: for *Charianthus* (Melastomataceae), a clade of six species in the Lesser Antilles all of which are hummingbird-pollinated (for species names see Supporting Information, Table S1), we used the nuclear ribosomal DNA internal transcribed spacer (i.e. the ITS region) alignment of Penneys & Judd (2005), which comprises 14 species, including all six bird-pollinated *Charianthus*, and 765 aligned positions. We added a sequence of *Mouriri* from GenBank (Supporting Information, Table S3) for cross-validation purposes. We used an ITS substitution rate of 1.44×10^{-9} substitutions per site year⁻¹ from *Empetrum*, Ericaceae (Kay, Whittall & Hodges, 2006). We chose this rate because *Empetrum* is a small shrub, as are species of *Charianthus*. The age inferred with this rate for the split (marked by a red dot in Supporting Information, Fig. S1a) between Melastomataceae and the clade to which *Mouriri* belongs was 37.36 (71.56–15.63) Mya. This overlaps with the age of 41.0 (28.0–58.0) Mya inferred by Bell, Soltis & Soltis (2010) for the same node. For the *Charianthus* chronogram see Supporting Information, Figure S1a.

For *Passiflora* subgenus *Decaloba* (Passifloraceae), which includes ten bird-adapted species in the West Indies (for species names see Supporting Information, Table S1, one of them, *Passiflora lancifolia* not closely related to the other hummingbird-pollinated species; see Supporting Information, Fig. S1b), we used the alignment of Krosnick *et al.* (2013), which comprises 796 aligned positions of ITS for 126 of the 230 species in this subgenus plus other Passifloraceae as outgroups. We used an ITS substitution rate of 8.34×10^{-9} substitutions per site year⁻¹, calculated for *Soldanella*, a long-lived herb (Kay *et al.*, 2006). The age inferred with this rate for the split between Passifloraceae and Paropsiaceae (marked by a red dot in Supporting Information, Fig. S1b) was 33.43 (25.96–50.37) Mya. This overlaps with the age of 28.0 (18.0–38.0) Mya inferred by Bell *et al.* (2010) for the same node. For the chronogram see Supporting Information, Figure S1b.

For *Goetzea* (Solanaceae), we used the alignment of Olmstead *et al.* (2008), which comprises 183 species representing all major lineages and most genera of Solanaceae, including two hummingbird-pollinated West Indian *Goetzea* species (for species names see Supporting Information, Table S1) and 3040 aligned positions of the plastid *ndhF* and *trnL-trnF* regions. For calibration, we used a normally distributed secondary constraint of 38.0 (29.0–47.0) Mya for the crown group of Solanaceae based on the age obtained for this node by Bell *et al.* (2010). The age inferred with this calibration for the stem age of the *Petunia* clade (marked by a red dot in Supporting Information, Fig. S1c) was 9.63 (4.63–16.19) Mya. This age overlaps with the age of 12.0 Mya inferred for the same node by Filipowicz & Renner (2012). For the chronogram see Supporting Information, Fig. S1c.

For *Cestrum* (Solanaceae), we used the alignment of Montero-Castro *et al.* (2006), which comprises 3492 aligned positions of the plastid *matK* gene and the *trnL-trnF* and *trnT-trnL* spacers for 32 of the 175 *Cestrum* species, plus five outgroups, four of them Solanaceae. Seven West Indian species are hummingbird-pollinated (species names in Supporting Information, Table S1) and all are in the DNA matrix. For cross-validation purposes we added a sequence of *Ipomoea purpurea* (Convolvulaceae). We used a plastid substitution rate of 7.0×10^{-10} substitutions per site year⁻¹ from Palmer (1991). The age inferred with this rate for the split between Convolvulaceae and Solanaceae (marked by a red dot in Supporting Information, Fig. S1d) was 53.57 (37.95–71.15) Mya. This overlaps with the age of 59.0 (49.0–68.0) Mya inferred by Bell *et al.* (2010) for the same node. For the chronogram see Supporting Information, Figure S1d.

For the Gesnerieae, a clade of four genera and 75 species distributed across the Antillean islands with a centre of diversity in the Greater Antilles and only three species in northern South America (Martén-Rodríguez & Fenster, 2010), we accepted the stem and crown ages of 26.0 and 8.5 Mya, respectively, inferred by Roalson, Skog & Zimmer (2008) under their preferred calibration scheme from a matrix that included 15 of the 75 species from three of the four genera. Martén-Rodríguez & Fenster (2010) enlarged this matrix to include 36 species from all four genera and supported the monophyly of the Antillean Gesnerieae.

For the *Poitea* clade (Fabaceae) we accepted the age of 9.3 Mya for *Poitea* and of 4.4 Mya for the five hummingbird-adapted species, listed in Supporting Information, Table S1, obtained under penalized likelihood by Lavin *et al.* (2003) from a matrix that included 12 of the 13 species of this genus.

For the Cuban hummingbird-pollinated species *Brunfelsia purpurea* (Solanaceae), we accepted the divergence time from its Cuban sister species *B. cestroides* of 3.5 Mya obtained by Filipowicz & Renner (2012). Their study included 18 of the 23 Antillean species and 19 of the 24 South American species of *Brunfelsia*.

BIOGEOGRAPHICAL RECONSTRUCTIONS

The distributions of hummingbird species were obtained from Birdlife International (2013) and those of the plants from Tropicos.org (<http://www.tropicos.org>) and relevant literature (e.g. Lavin *et al.*, 2003; Penneys & Judd, 2004, 2005; Filipowicz & Renner, 2012). We coded species for occurrence in one of the following six regions: South America, Central America, North America (north of the Trans-Mexican Volcanic Belt), Lesser Antilles, Greater Antilles and Bahamas. The Trans-Mexican Volcanic Belt was considered the border between North and Central America to separate tropical from temperate habitats. Ancestral area reconstruction relied on Bayesian binary MCMC analysis as implemented in RASP v.2.1beta (Yu, Harris & He, 2012). In this approach, the frequency of ancestral ranges at all nodes in the reconstruction is averaged over all input trees (in our case 1000 trees), allowing polytomies and very short branches (Yan, Harris & Xingjin, 2010). We ran the biogeographical MCMCs for 50 000 generations using the Jukes–Cantor + G model and sampling each 100 generations. No switches between areas or area combinations were forbidden. We only report ancestral areas that received $\geq 75\%$ support for one biogeographical region.

RESULTS

GEOGRAPHICAL ORIGINS AND DIVERGENCE TIMES OF THE WEST INDIAN HUMMINGBIRDS

Node ages for the focal species in the rate-calibrated 284 species tree and in the fossil-calibrated 221 species tree were almost identical, and we therefore show the more densely sampled chronogram from the 284 species tree (Fig. 1). The 14 endemic West Indian hummingbird species included in this chronogram belong to five lineages in mangoes, bee hummingbirds and emeralds. The only West Indian species missing from our sampling, *Mellisuga helenae*, occurs on Cuba and the adjacent Island of Youth, and unpublished data show that it is not sister to *M. minima* as expected from morphology (personal communication from C. W. Clark, Cornell Lab of Ornithology, in his October 2014 review of this paper).

The *Anthracothorax* clade, in which both species of the genus *Eulampis* are nested, split from its sister, *Chrysolampis mosquitus*, about 6.4 (5.5–7.3) Mya and colonized the Greater Antilles from South America (Figs 1 and 2A). Whether *Anthracothorax mango* colonized the Greater Antilles independently or whether a first *Anthracothorax* colonization was followed by a re-colonization around 4.6 (3.8–5.4) Mya cannot be determined from the present data. The common ancestor of *Anthracothorax dominicus*, *A. viridis*, *Eulampis jugularis* and *E. holosericeus* apparently colonized the Lesser Antilles from the direction of Venezuela (Fig. 2A).

The sister taxa *Calliphlox evelynae* from the Bahamas and *Mellisuga minima* from the Greater Antilles diverged from each other about 3.1 Mya and from *Archilochus* 3.3 (2.6–4.0) Mya (Fig. 1). Their common ancestor colonized the Greater Antilles and Bahamas either from North or from Central America. The three West Indian *Chlorostilbon* and *Cyanophaia bicolor* form a clade that diverged from its sister clade 6.4 (5.5–7.3) Mya and radiated in the West Indies around 4.9 (4.0–5.8) Mya (Fig. 1), having arrived from Central America (Fig. 2C). From the Greater Antilles, *Cyanophaia bicolor* colonized the Lesser Antilles around 4.5 Mya.

The common ancestor of the Jamaican streamertails (*Trochilus polytmus* and *T. scitulus*) diverged from a Central and South American *Amazilia* clade about 6.6 Mya, and the two species may have diverged from each other some 200 000 years ago (Fig. 1), although such young ages cannot be reliably inferred with our sparse data. Lastly, *Orthorhyncus cristatus*, a monotypic genus of emeralds, is sister to the South American *Stephanoxis/Anthocephala* clade and colonized the Lesser Antilles from South America about 5.7 (4.8–6.7) Mya (Figs 1 and 2E).

DIVERGENCE TIMES AND CLADE RICHNESS OF WEST INDIAN HUMMINGBIRD-ADAPTED PLANTS

Based on the criteria described in the Introduction and Materials and Methods, 101 West Indian plant species (90 of them endemic; see Supporting Information, Table S1) have flowers adapted to hummingbirds as pollinators. This number is a conservative minimum number that probably includes the oldest hummingbird-adapted species, given that trait evolution presumably took some time. Of these species, we were able to infer the ages of some 40 in eight clades in the families Fabaceae, Gesneriaceae, Melastomataceae, Passifloraceae and Solanaceae (Table 1, Supporting Information, Fig. S1a–d). *Brunfelsia purpurea* on Cuba and *Passiflora lancifolia* on Jamaica (Table 1), by comparison, adapted to hummingbird pollination recently. Figure 3 visualizes the stem and crown ages of the West Indian hummingbirds and the plant groups dated in this study.

DISCUSSION

The endemic West Indian hummingbird fauna is young compared with the much older hummingbird clades occurring in South America (McGuire *et al.*, 2014; Abrahamczyk *et al.*, 2014; our Fig. 1). The oldest West Indian (*in situ*) radiations date to the uppermost Miocene, between 5.7 and 6.6 Mya. The within-island speciation in the Jamaican streamertails, *Trochilus polytmus* and *T. scitulus*, has attracted considerable attention because it has occurred in one of the smallest oceanic island settings known (Gill, Stokes & Stokes, 1973; Lance *et al.*, 2009; McCormack *et al.*, 2012). We here date it to 200 000 years ago, fitting with the weak phylogeographical structure found with microsatellites by Lance *et al.* (2009) and with next-generation sequence data by McCormack *et al.* (2012). The second within-Caribbean radiation consists of the species of *Chlorostilbon* plus *Cyanophaia bicolor*, the third of *Anthracothorax* plus the two *Eulampis*, and the fourth of *Calliphlox evelynae* and *Mellisuga minima*. Our clock-dating effort is at the species level, not the population level and focuses on the West Indies, which is why we did not include the hairy hermit, *Glaucis hirsutus*, from tropical South America, Trinidad, Tobago and Grenada, at the fringe of the Caribbean basin. It is the sister species of *G. aeneus* from eastern Honduras, Panama, western Colombia and north-west Ecuador (McGuire *et al.*, 2014), and appears to have arrived in the West Indian region recently; clearly, this can only be inferred with population-level data.

The crown age of modern hummingbirds has been inferred to be 18.2 Mya (Bleiweiss, 1998), 26.0 Mya

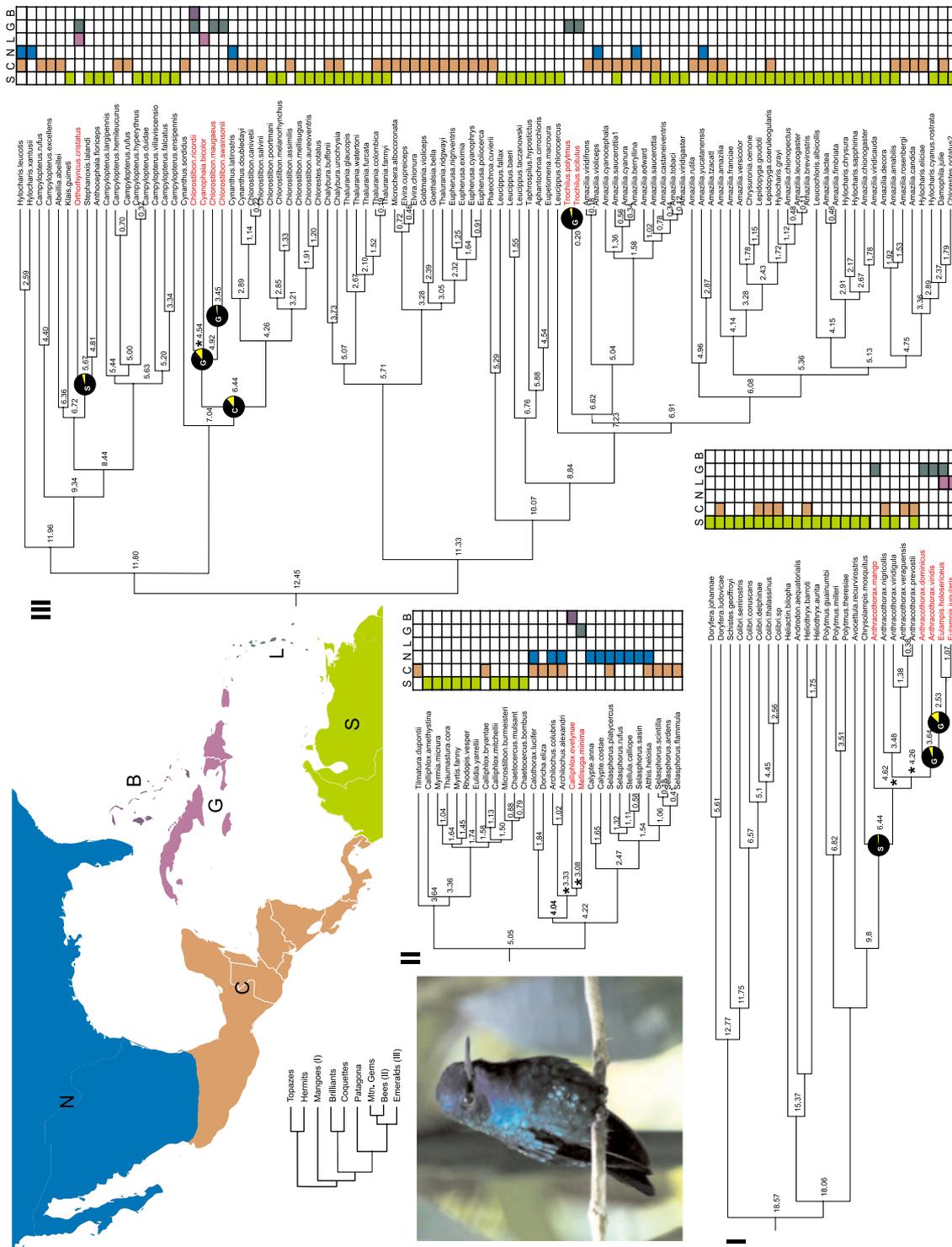


Figure 1. Chronograms for the hummingbird subfamilies with endemic species (in red) in the West Indies (I, mangoes; II, bee hummingbirds; III, emeralds) obtained from a relaxed clock model applied to mitochondrial and nuclear sequences. Numbers refer to node ages (Mya), and pie charts at internal nodes show the probabilities for alternative ancestral areas received by using a Bayesian binary MCMC analysis on 1000 Bayesian trees. Asterisks at nodes indicate nodes for which no single ancestral area received $\geq 75\%$ support. The grid column labels refer to: S, South America; C, Central America; N, North America; G, Greater Antilles; L, Lesser Antilles; B, Bahamas. The photo (by D. Comings) shows *Cyanophaea bicolor*, endemic to the Lesser Antilles.

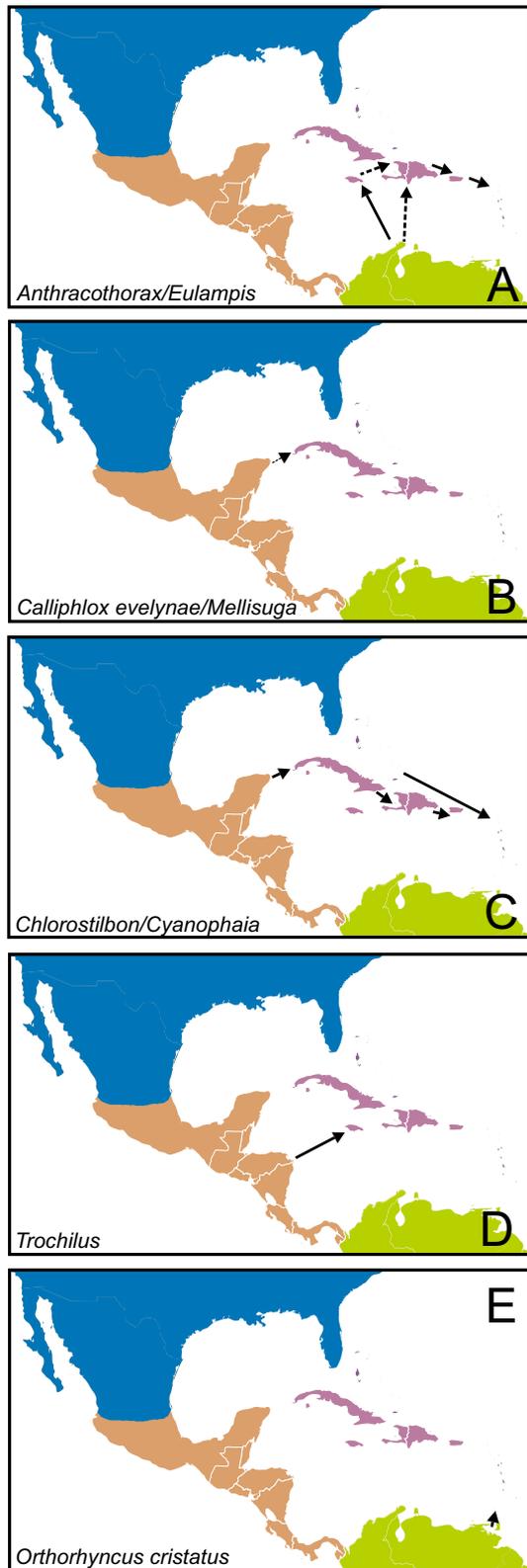


Figure 2. Colonization history of the endemic West Indian hummingbird lineages (A–E); arrows indicate direction of colonization inferred as described in Figure 1; dashed lines indicate ambiguous colonization directions.

(Jetz *et al.*, 2012), or 22.4 Mya (McGuire *et al.*, 2014). The oldest hummingbird clades and hummingbird-dependent plant clades in North America date to the Late Miocene, 5–9 Mya (S. Abrahamczyk & S. S. Renner, unpublished data), just like the hummingbirds and their coevolved plants in the West Indies, with neither partner producing impressive radiations (defined as more than ten species).

Colonization of the West Indies by hummingbirds must have involved the crossing of large expanses of ocean (Fig. 2). Hurricanes occasionally move living animals from South America northwards or from Central America eastwards (Censky, Hodge & Dudley, 1998; Hedges, 2001), but this explanation is not needed for hummingbirds because these birds actively fly long distances, even over open water, occasionally arriving in regions far from their typical distribution area (Bleiweiss, 1998; Williamson, 2001). The ancestor of the *Chlorostilbon/Cyanophaia* clade and probably also that of *Mellisuga minima/Calliphlox evelynae* appear to have colonized the Greater Antilles from Central America. For the latter, our ancestral area reconstruction is ambiguous (suggesting North America as equally likely); however, based on habitat preferences these birds are ecologically more similar to *Calothorax/Doricha* than to other temperate species (personal communication from C. W. Clark, Cornell Lab of Ornithology, in his October 2014 review of this paper). Colonization over, respectively, 560 or 640 km of ocean appears to explain the presence of *Anthracothorax* on the Greater Antilles and of *Trochilus* on Jamaica.

Comparison of the birds' stem and crown ages with the ages of the eight West Indian bird-adapted plant species and species groups reveals a general overlap (Fig. 3), similar to those of a study on the temperate zones of the Americas (S. Abrahamczyk & S. S. Renner, unpublished data). Our plant sampling includes the species-rich West Indian bird-pollinated radiations *Cestrum* (seven spp.), *Charianthus* (six spp.), Gesnerieae (75 species, c. 14 of them hummingbird-pollinated in the West Indies), *Passiflora* [ten species in one clade, but one, *P. penduliflora*, bat-pollinated (Kay, 2001), one in a separate clade] and *Poitea* (five spp.). We lack a dated phylogeny of *Ernodea* (seven spp.; Supporting Information, Table S1). There are no other equally large or larger West Indian bird-pollinated groups (Supporting Information, Table S1). Assuming that speciation takes time, the West Indian hummingbird-dependent 55 plant groups with fewer than seven species, not all of them endemic (Supporting Information, Table S1), may be younger than the radiations so far sampled, as indeed are the *Brunfelsia* and *Passiflora* species from Cuba and Jamaica included in our sample (Table 1). *Passiflora lancifolia* is pollinated by *Trochilus polytmus* (Tyrrell & Tyrrell,

Table 1. West Indian plant lineages with hummingbird-adapted flowers (Material and Methods), with geographical ranges and inferred stem and crown ages followed by 95% confidence intervals in parentheses; 95% confidence ranges are also shown in Fig. 1a–d and for Gesneriaceae and *Poitea* in the cited studies; Table S1 provides all species names

Species or clade	Family	No. of hummingbird-pollinated species (total species in the clade)	Distribution	Stem age (Mya)	Crown age (Mya)	Reference
<i>Brunfelsia purpurea</i>	Solanaceae	1 (1), 18 of 23 West Indian species in clock model	Greater Antilles	3.5	NA	Filipowicz and Renner (2012)
<i>Cestrum</i> Caribbean clade	Solanaceae	7 (7), all West Indian species in clock model, Fig. S1d	Greater Antilles	14.4	9.1 (3.2–15.4)	This study
<i>Charianthus</i>	Melastomataceae	6 (6), all species in clock model, Fig. S1a	Lesser Antilles	15.1	5.35 (1.2–13.5)	This study
Gesneriaceae	Gesneriaceae	8 (75 in 4 genera), 15 species in 3 genera in clock model	Greater and Lesser Antilles	26.0 (22.5–29.5)	8.5 (6.2–10.7)	Roalson <i>et al.</i> (2008)
<i>Goetzea</i> clade	Solanaceae	2 (2), both in clock model, Fig. S1c	Greater Antilles	3.1	2.3 (0.5–3.1)	This study
<i>Passiflora</i> Caribbean clade of subgen. <i>Decaloba</i>	Passifloraceae	8 (10); 9 in clock model Fig. S1b	Greater Antilles, Bahamas	2.8 (2.0–3.7)	2.5	This study <i>P. tulae</i> not shown in Fig. S1b, <i>P. penduliflora</i> bat-pollinated This study
<i>Passiflora lancifolia</i>	Passifloraceae	1 (1), in clock model, Fig. S1b	Greater Antilles	2.7	NA	This study
<i>Poitea</i> clade	Fabaceae	5 (13), 11 of 13 species in clock model	Greater and Lesser Antilles	9.3 (8.1–11.5)	4.4	Lavin <i>et al.</i> (2003)

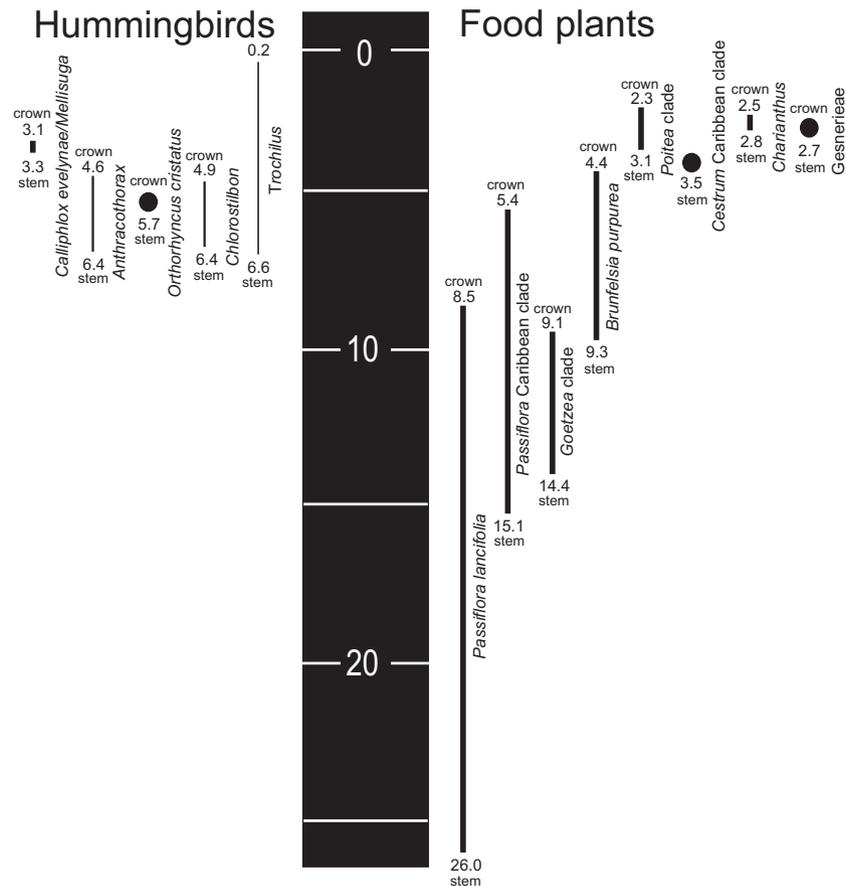


Figure 3. Stem and crown ages (Mya) of the independent West Indian hummingbird lineages or species (Fig. 1) and the eight hummingbird-adapted plant lineages or species dated in this study; 95% confidence intervals around bird estimates are given in the main text; those for plants are in Fig. S1 or Lavin *et al.* (2003: *Poitea*) and Roalson *et al.* (2008: Gesnerieae).

1990); the hummingbirds that visit *Brunfelsia purpurea* have been observed, but not identified (Filipowicz & Renner, 2012). West Indian hummingbirds, of course, also forage on flowers for which they are not the main pollinator (Martén-Rodríguez & Fenster, 2008; Martén-Rodríguez, Almarales-Castro & Fenster, 2009; Dalsgaard *et al.*, 2009, 2012), but these flowers will not possess the morphological and physiological features that constitute the hummingbird pollination syndrome and which indicate prolonged selection by hummingbird pollinators (Rosas-Guerrero *et al.*, 2014).

The crown group ages of the oldest and most species-rich plant groups, the West Indian *Cestrum* clade (9.1 Mya), Gesnerieae (8.5 Mya) and *Charianthus* (5.4 Mya), are unlikely to result from poor taxon sampling (see species numbers provided in Table 1), although error ranges around all clock estimates tend to be large. If true, these old ages might point to pollinator extinctions. The only West Indian hummingbird fossils are from the last glacial maximum in the Wisconsin Glacial Stage (Olson,

1982; Graves & Olson, 1987). There are also historical extinctions in the genus *Chlorostilbon* (Graves & Olson, 1987). Thus, hummingbird–plant mutualisms on individual islands may have been disrupted as suggested by Bleiweiss (1998: p. 94) who concluded, ‘If species composition has changed repeatedly over time on the islands, however, the contemporary flowers may not have evolved under the influence of contemporary hummingbird species. Thus, coevolution may be said to have occurred only at the level of the size categories, but may have involved species other than those currently participating in the system.’

CONCLUSIONS

Over the past 6 Myr, hummingbirds have repeatedly colonized the West Indies from Central and South America. These introductions have produced four small radiations with two to four species. On the plant side, the most species-rich bird-pollinated West Indian groups – Gesnerieae and clades of *Passiflora*,

Cestrum, *Charianthus* and *Poitea* – also have not produced any conspicuous radiations, probably because small islands provide too few opportunities for geographical isolation, especially in bird-pollinated plants, where pollen flow may be relatively extensive and far-reaching. The overlap between the crown group ages of West Indian hummingbirds and nectar plants showing clear evidence of selection by bird pollinators is relatively poor (Fig. 3), which may be due to insufficient species sampling or local bird extinctions followed by re-colonization, a possibility that merits further study.

ACKNOWLEDGEMENTS

We thank S. Bellot, F. Antunes Carvalho and R. E. Ricklefs for advice, K. Porter-Utley for providing the *Passiflora* alignment, D. Comings for the permission to use the photo of *Cyanophaia bicolor*, and C. Clark and another anonymous reviewer for helpful comments.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Plant chronograms.

Table S1. The 101 hummingbird-adapted species (in 48 genera) occurring in the West Indies (90 of them endemic). E, endemic; N, non-endemic. Groups with four or more species are highlighted in bold. Species included here have one or more of the following traits: (i) scentless brightly coloured, often red flowers with (ii) elongate corolla tubes, (iii) large amounts of sucrose-rich nectar, (iv) no landing platform, (v) diurnal opening, and (vi) stigmas and stamens placed such that foraging hummingbirds effectively cause cross-pollination.

Table S2. GenBank accession numbers of the newly sequenced hummingbird species.

Table S3. GenBank numbers of the species added to Convolvulaceae and Melastomataceae alignments.