ORIGINAL ARTICLE

Evolutionary flexibility in five hummingbird/plant mutualistic systems: testing temporal and geographic matching

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ABSTRACT

Aim Partners in co-evolutionary interactions must be ecologically interdependent and at least at some stage have matching traits and more or less overlapping ranges. As co-evolution is a process, neither the mutual dependence nor the trait and range matching are expected to be static or perfect. Here we investigate the extent of evolutionary flexibility in tight pollination mutualisms between hummingbirds and plants, ranging from straight-billed species to sicklebills.

Location The Americas.

Methods The five considered pollination mutualisms are between the following hummingbird and plant species: *Calypte anna* and *Ribes speciosum* (Grossulariaceae); *Basilinna xantusii* and *Arbutus peninsularis* (Ericaceae); two species of *Sephanoides* and *Tristerix aphyllus/corymbosus* (Loranthaceae); two species of *Eutoxeres* and 34 species of curved-corolla *Centropogon* (Campanulaceae); and six species of *Oreotrochilus* and seven species of *Chuquiraga* (Asteraceae). While ecological interdependence and trait matching in these mutualisms are well established, geographic occurrence data and molecular clock-based ages for the 10 clades were newly compiled.

Results We found matching bird and plant stem ages in two of the five systems and (much) older bird than plant ages in the other three. The implied adaptation of plant populations to already existing hummingbird species fits with the modelled distributions. In three of the systems, the ranges of the plants overlap those of their pollinators by > 90%; conversely, the range overlap between the bird species and the plant species they pollinate is much less than that. Surprisingly, the age mismatch was greatest in the *Eutoxeres/Centropogon* system, in spite of the perfect morphological fit among bills and corollas, illustrating the evolutionary flexibility of these mutualisms and the apparently rapid 'addition' of further plant species.

Main conclusions These findings illuminate the macroevolutionary assembly of hummingbird/plant mutualisms, which has been highly dynamic, even in specialized systems with perfect morphological trait fitting.

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INTRODUCTION

The co-evolutionary pollination mutualisms between hummingbirds and their food plants are both tight (Stiles, 1973, 1975, 1979, 1981, 1985; Temeles & Kress, 2003; Temeles *et al.*, 2009) and evolutionarily flexible, meaning that partners broaden or narrow the range of species they successfully interact with for food, in the case of the birds, or gamete transport, in the case of the plants (Tripp & McDade, 2013; Abrahamczyk *et al.*, 2014; Abrahamczyk & Renner, 2015).

Hummingbirds take nectar from a range of plants, and most hummingbird-pollinated plant species employ a range of hummingbirds as pollinators (summarized in Schuchmann, 1999; Baza Mendonça & dos Anjos, 2013), even though close morphological matching between flower shapes and hummingbird bills increases efficiency in hummingbirds' nectar foraging (Maglianesi *et al.*, 2014). The breadth of most hummingbirds' foraging niches is also evident from the asymmetry between *c.* 7000 plant species being morphologically adapted to pollination by 'just' 365 species of hummingbirds (Abrahamczyk & Kessler, 2015). Studies that rely on molecular clock dating of interacting groups of plants and hummingbirds suggest that, within one biogeographic region, the phylogenetically oldest hummingbird lineage and its food plants have similar ages, while other plant groups later shifted to hummingbird pollination over evolutionary time (Abrahamczyk *et al.*, 2015: West Indies; Abrahamczyk & Renner, 2015: temperate North and South America). Exploratory visits by hummingbirds looking for nectar must have provided the starting points for new co-evolutionary relationships, while earlier relationships dissolved due to habitat changes, which may have altered the relative abundances of the birds or the plants. This probably occurred, e.g. in the morphologically well-matched bill and flower shapes of the Sword-billed hummingbird *Ensifera ensifera* and certain passion-flower species (*Passiflora* section *Tacsonia*). *Ensifera* bills and *Tacsonia* corolla tubes have lengths of up to 10 cm and perfectly match each other. In spite of this morphological matching, *Ensifera* dependence was lost several times, with *Tacsonia* species shifting to pollination by bats or shorter-billed birds (Abrahamczyk *et al.*, 2014).

During parts of the year, some hummingbird species depend on just a few or even one species of plant as their main nectar source (Abrahamczyk & Kessler, 2014). Pairs of such interdependent bird and plant species tend to occur at latitudes outside the tropics, on islands or at high elevations in the tropics (Stiles, 1973; Carpenter, 1978; Ortiz-Crespo & Bleiweiss, 1982; Arriaga *et al.*, 1990; Woods *et al.*, 1998; Temeles *et al.*, 2000; Aizen, 2005; Abrahamczyk & Renner,

2015). We focus on five such extremely narrow interactions to investigate how old they are, and which of the partners drove the interaction, meaning which partner first possessed key traits to which the other then adapted, as posited in the pollinator shift hypothesis (Wasserthal, 1997; Whittall & Hodges, 2007). The studied clades span a range of biogeographic regions, ecosystems and bill/corolla tube morphologies (Table 1; Stiles, 1973; Carpenter, 1978; Ortiz-Crespo & Bleiweiss, 1982; Stein, 1987, 1992; Arriaga *et al.*, 1990; Woods *et al.*, 1998; Aizen, 2005). For each system, we compiled bird and plant chronograms and occurrence-based range maps. We expected closer matching of geographic ranges and divergence times in pollination systems involving species with extreme bill and flower morphologies (Abrahamczyk *et al.*, 2014) than in mutualisms involving short- to medium-sized straight bills and corollas.

MATERIALS AND METHODS

The framework

A review of the feeding ecology of hummingbirds found five extremely narrow hummingbird/plant mutualisms (Abrahamczyk & Kessler, 2014). In these systems, a particular plant species is the only nectar source for a hummingbird species for certain weeks, often during winter, and this plant depends almost exclusively on that hummingbird for its pollen transport (Table 1: Stiles, 1973; Carpenter, 1978; Ortiz-Crespo & Bleiweiss, 1982; Stein, 1987, 1992; Arriaga *et al.*, 1990; Woods *et al.*, 1998; Aizen, 2005). For the two Sicklebill species, *Eutoxeres aquila* and *E. condamini*, the *Centropogon granulosus* group with *c.* 34 species (Stein, 1987), all with abruptly curved corollas and flowers borne on sturdy inflorescences (see Fig. S1.1 in Supporting Information), provides an exclusive food source that matches with *Eutoxeres* being a perch-feeder (Gill, 1987). However, some *Centropogon* species are not monophyletic (Lagomarsino *et al.*, 2014). Geographic information for all involved species is given below.

Table 1 Hummingbird/plant mutualisms of species/clades that depend on each other during a couple of weeks or months for food and pollination, their corolla and bill morphology and distribution.

Hummingbird species/clades	Plant clade	Corolla & bill morphology	Geographic origin	Reference for the interaction
Basilinna (Hylocharis) xantusii	*Arbutus peninsularis* (Ericaceae)	Medium-sized, straight	Baja California	Arriaga *et al.* (1990)
Calypte anna	*Ribes speciosum* (Grossulariaceae)	Medium-sized, straight	Western California	Stiles (1973)
Eutoxeres (two species)	*Centropogon granulosus* clade (Campanulaceae; 34 species)	Long, strongly curved	Northern to central Andes	Stein (1987, 1992)
Oreotrochilus (seven species)	*Chuquiraga spinosa* clade (Asteraceae; eight species)	Medium-sized, straight	Northern to central Andes	Carpenter (1978)
Sephanoides (two species)	*Tristerix aphyllus/corymbosus* (Loranthaceae)	Short, straight	Temperate South America & Juan-Juan-Fernández Islands	Aizen (2005)

Sequencing of plants and compiling plant chronograms

Chronograms for *Ribes, Arbutus* and *Tristerix* were taken from Abrahamczyk & Renner (2015) and that for *Centropogon* from Lagomarsino *et al.* (2016). To generate a phylogeny and chronogram for *Chuquiraga* (Asteraceae), we modified the Barnadesioideae alignment of Gruenstaeudl *et al.* (2009) by adding *Chuquiraga* sequences from GenBank and generating 87 additional sequences from herbarium material, resulting in a matrix of four chloroplast markers: *matK* with parts of the *trnK* intron, the *rpoC1* intron, the *psbA–trnH* intergenic spacer, and the *trnL* intron and *trnL–trnF* intergenic spacer. For sequencing we used the same protocol as the original publications (Panero & Funk, 2008; Gruenstaeudl *et al.*, 2009). All plant sources with their voucher information are listed in Table S1.1. The final phylogeny comprises 5224 aligned nucleotides and 46 taxa, including 19 of the 27 species of *Chuquiraga*. Six of the eight missing *Chuquiraga* species come from the insect-pollinated section *Acanthophylla*, which is less relevant for this study (Padin *et al.*, 2015). Dating relied on BEAST 1.8.1. (Drummond & Rambaut, 2007), with a relaxed clock model (ulcd.stdev 0.844), calibrated with a Mutisioideae pollen fossil from Patagonia (*Mutisiapollis telleriae*) dated to 47.5 million years ago (Ma) (Barreda *et al.*, 2010), which was assigned to the split between Mutisioideae/Wunderlichioideae and Barnadesioideae. For this constraint, we used a gamma distribution with an off-set at 47.5 Ma, a shape parameter of 1.0 and a median of 48.35, allowing 95% of the ages to fall between 47.53 and 52 Ma, and 2.25% to be older than 52 Ma. We employed a Yule tree prior as recommended for species-level analyses (BEAST manual) and used a GTR+G substitution model with four rate categories. Markov chain Monte Carlo (MCMC) chains were run for 70 million generations, sampling every 10,000th generation. Effective sampling sizes were above 300 as recommended in the BEAST manual. Of the posterior 7000 trees, we dropped the first 25% as burn-in and then checked for convergence using TRACER 1.5 (Rambaut & Drummond, 2007). Error bars (95% confidence intervals) are only shown for notes having a posterior probability ≥ 98%. Maximum clade credibility trees were edited in FIGTREE 1.3.1.

Sequencing of hummingbirds and generating chronograms

We newly sequenced two mitochondrial [*NADH dehydrogenase* subunits 2 and 4; ND2 and ND4, together 1994 base pairs (bp)] and two nuclear markers [intron 7 in the *beta fibrinogen* (BFib) gene and intron 5 in the *adenylate kinase* (AK1) gene, 2045 bp] of *Oreotrochilus leucopleurus,* CUMV Bird 55860 from the Cornell Museum of Vertebrates in Ithaca, NY, collected near Jujuy, Argentina, in 2005. For DNA extraction and amplification, we followed McGuire *et al.* (2014). Table S1.2 provides the GenBank accession numbers. The new sequences were added to the hummingbird alignment of Abrahamczyk & Renner (2015), resulting

in an alignment of 284 hummingbird and five swift species (as outgroup) and 4039 aligned positions. We again used the BEAST software, and, as in Abrahamczyk & Renner (2015), we applied a strict clock model (ucld.stdev value of a relaxed clock = 0.193) calibrated with a hummingbird stem group fossil, *Paragornis messelensis,* found in the oil shale of Messel in Southern Germany (Mayr, 2003). The results from relaxed clock models and other calibrations are similar as discussed in our earlier study. The Messel fossil provides a minimum age for the divergence between swifts and hummingbirds of 47.5 Ma (Mayr, 2009). For further details of the dating see Abrahamczyk & Renner (2015).

Geographic distributions and phylogenetic age comparisons

The distribution of hummingbirds was obtained from Birdlife International (BirdLife International & NatureServe, 2015). The distribution of the plant clades came from published distribution maps and GBIF data (www.gbif.org) in combination with expert knowledge (see Table S1.3). To reduce the impact of identification errors in GBIF (Goodwin *et al.*, 2015), we downloaded data only for easily recognizable taxa, such as the *Centropogon granulosus* species group as a whole (*c.* 34 species with extremely curved corollas; Stein, 1987), or *Arbutus peninsularis*, the only species of this genus in Baja California. All GBIF data were critically revised and duplicated records or obviously wrongly georeferenced specimen (e.g. records in Amazonian lowland of exclusively Andean plant species) were removed. For the *Chuquiraga spinosa* species group, we focused on occurrence data from specimens identified by C. Ezcurra, an expert on *Chuquiraga*, as well as the type localities of the narrow endemics *Chuquiraga arcuata* and *C. raimondiana* (Ezcurra, 1985; Harling, 1991; Granda Paucar, 1997).

To compare the fit of the distribution ranges of hummingbird species and their mutualistic plant species and vice versa, we modelled the suitable abiotic niches of the plant clades first, using MAXENT 3.3.3k (Phillips *et al.*, 2006; Phillips & Dudik, 2008), relying on the default settings. MAXENT includes a range of feature types, and subsets of these can be used to simplify the solution. By default, the program uses simpler models if few samples (< 80) are available (Elith *et al.*, 2011). Our distribution models are based on 110–1200 samples, except for the model for the small-ranged *Arbutus peninsularis*, which is based on seven samples. We used the CHIRPS climate data set (BIO1–BIO19), which has a spatial resolution of 3 by 3 Arcminutes (approximately 5.5 km × 5.5 km at the equator; Deblauwe *et al.*, 2016). Distribution ranges of *Tristerix aphyllus/T. corymbosus* and the *Centropogon granulosus* and *Chuquiraga spinosa* species groups were modelled by combining the coordinates of specimens belonging to the respective clades. Potential distribution area was chosen based on the 90% threshold provided by MAXENT. Unlikely predicted distributions, remote from the input coordinates (e.g. distribution in Venezuelan Tepuis for Andean species) were removed from the distribution area. The sizes of distribution areas for both, plant and bird species, were

calculated in Esri ArcMap™ 10.2.0.3348, using South America and North America-Albers-Equal-Area-Conic projection for the respective hemispheres. In a second step, we compared the fit of the distribution ranges (in km²) of the individual hummingbird species and their mutualistic plant species/clades and vice versa in ArcMap. Percentages of overlap were calculated in MS Excel 2013.

To evaluate the overlap between the phylogenetic ages of the bird and plant species/clades, we compared the relevant 95% confidence intervals. For nodes in our bird phylogeny that lack confidence intervals (because the respective branches are too short for beast to calculate a 95% confidence interval around the node age), we additionally used the confidence intervals given in McGuire *et al.* (2014; our Table 2).

RESULTS

Plant divergence times

We inferred a crown group age of Barnadesioideae of 37.4 Ma (95% HPD: 25.6–48.8 Ma; see Fig. S1.2), which

agrees well with the age for the same node (30–35 Ma) inferred by Kim *et al.* (2005). Within *Chuquiraga*, we identified two evolutionary switches from insect to hummingbird pollination: one encompassing the *Chuquiraga spinosa* species group, which contains almost all hummingbird-pollinated species, and a second containing just *C. longiflora* (2.97 Ma), which is part of an otherwise insect-pollinated clade (Fig. 1). The age of the *Chuquiraga spinosa* species group is 10.81 Ma (95% HPD 5.2–17.39 Ma; crown age 7.1 Ma), which fits the stem age of the *Oreotrochilus* species that pollinate these plants (6.8 Ma; Table 2). Clade or species ages for *Ribes, Arbutus, Tristerix* and *Centropogon* are listed in Table 2.

Hummingbird divergence times

For *Oreotrochilus,* we found a stem age of 6.8 Ma and a crown age of 1.62 Ma, but statistical support for the respective nodes is weak (see Fig. S1.3). The newly sequenced *Oreotrochilus leucopleurus* from Argentina is sister to an *O. estella* individual from Peru, and both form the sister clade to the remaining species of *Oreotrochilus*. Divergence times

Table 2 Stem ages of hummingbird/plant mutualisms; hummingbird ages in bold are newly inferred in this study; hummingbird ages in standard letters are from McGuire *et al.* (2014).

Hummingbird clade stem age [age, 95% HPD (Ma)]	Plant clade stem age [age, 95% HPD (Ma)]	Ages and distribution fitting?	Reference for plant clade age
Basilinna (*Hylocharis*) *xantusii* (**2.75; 2.11–3.45**/2.59; 1.78–3.41)	*Arbutus peninsularis* (Ericaceae) (0.8; –)	No/No	Abrahamczyk & Renner (2015)
Calypte anna (**2.17; 1.69–2.66**/1.67; 1.3–2.22)	*Ribes speciosum* (Grossulariaceae) (1.5; 0.14–3.27)	Yes/No	Abrahamczyk & Renner (2015)
Eutoxeres (**21.45; 18.18–24.97**/21.48; 19.44–23.7)	*Centropogon granulosus* clade Campanulaceae; (a) (3.64; 2.27–10.1) (b) (2.26; 1.64–2.69)	No/No	(a) Antonelli (2009) (b) Lagomarsino *et al.* (2016)
Oreotrochilus (**6.8; –**/7.78; 6.85–8.7)	*Chuquiraga spinosa* clade Asteraceae; (10.81; 5.2–17.39 – Crown age: 7.1; –)	Yes/Yes	This study
Sephanoides (**14.29; –**/13.33; 11.93–14.74)	*Tristerix aphyllus/corymbosus* (Loranthaceae) (4.7; 1.88–7.72)	No/No	Abrahamczyk & Renner (2015)

Figure 1 Molecular clock-dated phylogenies of *Chuquiraga* (a) and *Oreotrochilus* (b). Numbers at nodes give the inferred ages in million years. Hummingbird-pollinated *Chuquiraga* and *Oreotrochilus* species in bold. Photo of *Oreotrochilus chimborazo* at *Chuquiraga jussieui* by Joseph C. Boone, www.wikipedia.org.

of *Calypte anna*, the two species of *Eutoxeres, Basilinna* (*Hylocharis*) *xantusii* and the species of *Sephanoides* are shown in Table 2.

Overlap of geographic ranges and phylogenetic ages between birds and plants

The distribution ranges of *Ribes speciosum, A. peninsularis, T. aphyllus* and *T. corymbosus* overlap the ranges of their mutualistic hummingbirds by > 90%. The ranges of *Calypte anna, Basilinna* (*Hylocharis*) *xantusii* and the two species of *Sephanoides* overlap the ranges of *R. speciosum, A. peninsularis, T. aphyllus* and *T. corymbosus* by ≤ 47% (Fig. 2; Table 3). For the *Centropogon granulosus* species group and the two *Eutoxeres* species as well as the *Chuquiraga spinosa* species group and the relevant *Oreotrochilus* species, the mutual range overlaps were between 38% and 63% (Fig. 2, Tables 3 & 4). *Oreotrochilus* species occurring in the northern part of the distribution range of the genus (*O. chimborazo, O. melanogaster* and *O. stolzmanni*) strongly overlap the range of the *C. spinosa* species group (≥ 79%), while species occurring in the southern part of the range (*O. adela, O. leucopleurus* and *O. estella*) barely overlap that range (≤ 44%; Table 4). For the *Calypte anna/R. speciosum* and *Oreotrochilus/Chuquiraga spinosa* species groups, we found matching ages of both partners, while for the *Eutoxeres/Centropogon granulosus, Basilinna xantusii/Arbutus peninsularis* and *Sephanoides/Tristerix aphyllus/corymbosus* species groups, we found that the birds were older than the plants (Table 2).

DISCUSSION

In this study, we compared the phylogenetic ages and distribution ranges of five ecologically narrow pollination mutualisms: Four of these involve hummingbirds with short- to medium-sized, straight bills and plants with matching flower tubes (Fig. 1) that are mutually dependent on each other during a few weeks each year, and the fifth involves hummingbird species with extremely curved bills and plants with fitting corolla shapes. Given that our hummingbird chronogram includes 284 of the 361 known hummingbird species, the problem of artificially long branches, and hence overestimated divergence times, due to missing species should be relatively minor. In the interactions involving straight-billed hummingbirds, namely *Calypte anna* and *R. speciosum*, and *Basilinna* (*Hylocharis*) *xantusii* and *Arbutus peninsularis*, both in south-western North America, *Sephanoides* and *Tristerix aphylla/corymbosa* in Patagonia, and *Oreotrochilus* and the *Chuquiraga spinosa* species group in the Andes, the respective hummingbirds are key pollinators during winter when no other nectar resources are available to them (Stiles, 1973; Arriaga *et al.*, 1990; Aizen, 2005). Except in the *Oreotrochilus/Chuquiraga* interactions, all these mutualisms involve plant species that are younger (by 0.6–9.5 Ma) and have smaller potential ranges than their pollinating hummingbirds (Fig. 2). The closest relatives of most are insect-pollinated

(the exception being *Tristerix aphylla/corymbosa*, the relatives of which are hummingbird-pollinated), and the shifts to hummingbird pollination seem to have involved one-sided morphological adaptation of the plants to the birds. The involved hummingbird populations of *Calypte anna* and *Sephanoides sephanoides* seem to have changed if not in bill morphology, then at least in behaviour. They stopped migrating, while populations of *Calypte anna* and *Sephanoides sephanoides* that do not co-occur with these plant species still migrate (Schuchmann, 1999).

In the *Oreotrochilus/Chuquiraga spinosa* system, the distribution ranges and stem ages match, but crown ages do not; the hummingbirds are about 1.62 Ma (95% HPD: 1.2–2.05 Ma) and the plants about 7.1 Ma old (Table 2). These groups may first have encountered each other in the zone above timberline during the uplift of the northern and central Andes 6–10 Ma ago (Gregory-Wodzicki, 2000). The *Chuqiraga spinosa* species group seems to have expanded into the high central and northern Andes from the southern temperate Andes (Ezcurra, 2002), while *Oreotrochilus* may have originated in the northern Andes as suggested by the distribution of its closest relatives, *Polyonymus caroli* and *Opisthoprora euryptera*, which occur at elevation of 1500–3600 m (Schuchmann, 1999). *Oreotrochilus* may have begun colonizing elevations up to 5200 m in co-evolution with the *Chuqiraga spinosa* species group. By parallel adaptation to the harsh environmental conditions of their new habitat with extremely thin air and strong frost during night, the ancestors of both clades diverged from their closest relatives, with the *Chuqiraga spinosa* species group then radiating from about 5.9 Ma onwards. *Oreotrochilus* started radiating more recently, perhaps due to population fragmentation within its expanding range. The ranges of the northern species (*O. chimborazo, O. stolzmannii* and *O. melanogaster*) strongly overlap with the *Chuquiraga spinosa* species group (> 79%; Table 4) on whose nectar these birds depend (Carpenter, 1978; Ortiz-Crespo & Bleiweiss, 1982), but the southern species or populations (*O. adela, O. leucopleurus* and southern populations of the non-monophyletic *O. estella*) barely overlap with the *Chuquiraga spinosa* species group (≤ 44%; Table 4), and these birds instead mainly feed on species of Asteraceae, Bromeliaceae, Lamiaceae and Loranthaceae (Schuchmann, 1999).

In the sicklebill (*Eutoxeres*) system, which comprises two species of hummingbirds with the same type of extremely curved bills and numerous species of *Centropogon* with abruptly curved corolla tubes that depend on sicklebills as their main or sole pollinators (Stiles, 1985; Stein, 1987; Volpe, 2014), we found the largest temporal mismatch: *Eutoxeres* is about 21.45 Ma (95% HPD: 18.18–24.97 Ma) old, the *Centropogon* clade only 3.64 Ma (95% HPD: 2.27–10.1 Ma). Species boundaries in the *Centropogon granulosus* clade, represented by 33 accessions in the study of Lagomarsino *et al.* (2014), are problematic, with eight accessions named *C. 'granulosus'* appearing in six positions throughout the eucentropogonid clade. Several other species are equally
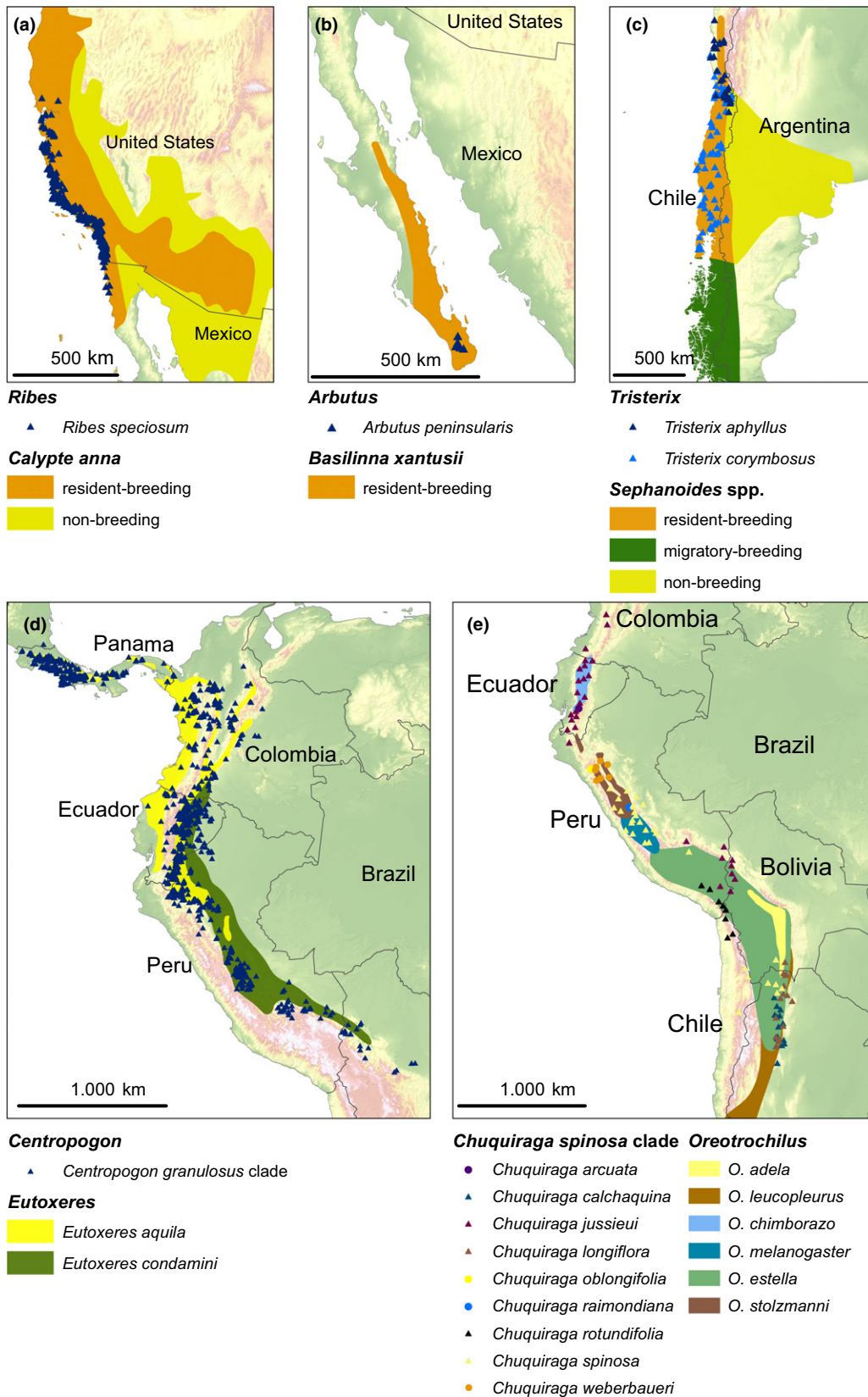
Figure 2 Modelled suitable distribution ranges of the hummingbirds and the plants depending on them for pollination. (a) *Calypte anna/Ribes speciosum*, (b) *Basilinna (Hylocharis) xantusii/Arbutus peninsularis*, (c) *Sephanoides/Tristerix aphyllus/corymbosus*, (d) *Eutoxeres/Centropogon granulosus* clade and (e) *Oreotrochilus/Chuquiraga spinosa* clade.

Table 3 Range overlaps between hummingbirds and plants on whose nectar they depend almost exclusively during at least part of the year.

Mutualistic partners	Total area (1000 km²)	Area of co-existence (1000 km²)	Co-existence (%)
Calypte anna (resident) with *Ribes speciosa*	493.1	28.8	6
Calypte anna (non-breeding) with *Ribes speciosa*	777	0	0
Ribes speciosa with *Calypte anna* (resident)	28.8	28.8	100
Basilinna (*Hylocharis*) *xantusii* with *Arbutus peninsularis*	41.9	4.6	11
Arbutus peninsularis with *Basilinna* (*Hylocharis*) *xantusii*	4.6	4.6	100
Sephanoides spp. (resident-breeding) with *Tristerix aphyllus/corymbosus*	252.1	119.3	47
Sephanoides spp. (non-breeding) with *Tristerix aphyllus/corymbosus*	550.2	2.3	0
Sephanoides ssp. (breeding) with *Tristerix aphyllus/corymbosus*	302.2	0.1	0
Tristerix aphyllus/corymbosus with *Sephanoides* spp. (resident)	132.4	121.7	92
Eutoxeres spp. with *Centropogon granulosus* species group	610.2	367.3	60
Centropogon granulosus species group with *Eutoxeres* spp.	664.5	367.3	55
Oreotrochilus spp. with *Chuquiraga spinosa* species group	1055	400.3	38
Chuquiraga spinosa species group with *Oreotrochilus* spp.	630.7	400.3	63

Table 4 Range overlaps of *Oreotrochilus* species with the *Chuquiraga spinosa* species group.

	Total area (km²)	Area of co-existence (km²)	Co-existence (%)
Oreotrochilus spp.	1055	400.3	38
Oreotrochilus adela	50.6	19.7	39
Oreotrochilus chimborazo	36.9	32.1	87
Oreotrochilus estella	595.8	263.1	44
Oreotrochilus leucopleurus	404.1	80.9	20
Oreotrochilus melanogaster	55.3	43.5	79
Oreotrochilus stolzmanni	61.4	55.8	91

para- and polyphyletic. This may be an indication of hybridization (Lagomarsino *et al.*, 2014), brought about by sharing the same pollinator species. While the *Centropogon* species with the abruptly curved corollas depend on sickle-bills for their gamete transport (see Fig. S1.1), both *Eutoxeres* species also visit species of *Heliconia* with curved corollas (Stiles, 1975, 1981; Gill, 1987). Different from *Centropogon*, *Heliconia* is an old genus [crown age 38.5 Ma (95% HPD 21.7–46.2 Ma); Iles *et al.*, 2016]. This implies that *Heliconia* flowers were available when sicklebills evolved and that both partners selected on each other's morphology as they still do (Temeles & Kress, 2003; Temeles *et al.*, 2009).

While *Heliconia* and *Eutoxeres* may have co-evolved for more than 20 Ma, *Centropogon* adapted its corollas to today's *Eutoxeres* species only over the past 2–3 Ma, during the period of fastest uplift of the Columbian Andes (Gregory-Wodzicki, 2000). The availability of a reliable food resource allows *Eutoxeres* to inhabit elevations > 2000 m, which would not be possible if these birds only relied on *Heliconia*, which does not tolerate cold weather and is confined to the lowlands (Andersson, 1989). This dependence on two main food resources, *Centropogon* at higher elevations and *Heliconia* at lower elevation, explains the only partial (55%) overlap of the distribution ranges of *Eutoxeres* and

the *Centropogon granulosus* species group (Fig. 2d). The asymmetric dependency of *Eutoxeres/Centropogon* can be compared to the mutualisms between the Sword-billed hummingbird (*Ensifera ensifera*) and a clade of 62–64 species of *Passiflora* (all in section *Tacsonia*) studied earlier (Abrahamczyk *et al.*, 2014). In the *Ensifera/Passiflora* system, we found that the plant clade dates to 10.7 Ma, the bird to 11.6 Ma, while in the *Eutoxeres/Centropogon* case, the birds may be much older than *Centropogon*. In both systems, we found 'escapes' away from strict dependence on *Ensifera* or *Eutoxeres* pollination. In the *Passiflora–Tacsonia* clade, a few species have shifted to pollination by short-billed hummingbirds and bats; in the *Centropogon* clade, two species, *C. amplicorollinus* and *C. cuatrecasanus*, have switched to pollination by other hummingbirds (Stein, 1987). Whether these shifts occurred independently is unknown because the species have not yet been sequenced.

CONCLUSIONS

Our expectation that at least some of the five mutualistic systems studied here would be at a stage of completely matching ranges and ages (divergence times) was not met. Instead, all were at stages where partners had switched more or less recently. Nevertheless, at least some bird populations currently rely on 'their' recently acquired nectar sources in winter and have abandoned migration, and others could not occur at high elevations. These results illuminate the macroevolutionary assembly of hummingbird/plant mutualisms, which has been highly dynamic, even in very specialized systems.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Supplementary figures and tables.

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