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

Stefan Abrahamczyk1* and Constantin Poretschkin1 and Susanne S. Renner2

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 xantusi* 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.

Keywords Andes, *Centropogon*, *Chuquiraga*, co-evolution, distribution ranges, *Eutoxeres*, *Oreotrochilus* hummingbirds, pollination

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 evolutionary 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; 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.

### 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.

<table>
<thead>
<tr>
<th>Hummingbird species/clades</th>
<th>Plant clade</th>
<th>Corolla &amp; bill morphology</th>
<th>Geographic origin</th>
<th>Reference for the interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basilinna (Hylocharis) xantusi</td>
<td>Arbutus peninsularis (Ericaceae)</td>
<td>Medium-sized, straight</td>
<td>Baja California</td>
<td>Arriaga et al. (1990)</td>
</tr>
<tr>
<td>Calypte anna</td>
<td>Ribes speciosum (Grossulariaceae)</td>
<td>Medium-sized, straight</td>
<td>Western California</td>
<td>Stiles (1973)</td>
</tr>
<tr>
<td>Eutoxeres (two species)</td>
<td>Centropogon granulosus clade (Campanulaceae; 34 species)</td>
<td>Long, strongly curved</td>
<td>Northern to central Andes</td>
<td>Stein (1987, 1992)</td>
</tr>
<tr>
<td>Oreotrochilus (seven species)</td>
<td>Chuquiraga spinosa clade (Asteraceae; eight species)</td>
<td>Medium-sized, straight</td>
<td>Northern to central Andes</td>
<td>Carpenter (1978)</td>
</tr>
<tr>
<td>Sephanoides (two species)</td>
<td>Tristerix aphyllus/corymbosus (Loranthaceae)</td>
<td>Short, straight</td>
<td>Temperate South America &amp; Juan-Juan-Fernández Islands</td>
<td>Aizen (2005)</td>
</tr>
</tbody>
</table>
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 (Mutisialpolis 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 1.5 and Barnadesioideae alignment of Gruenstaeudl et al. (2009) by modifying the 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 checked for convergence using 300 as recommended in the every 10,000th generation. Effective sampling sizes were above (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 Oreotrichulus leucopaeactus, 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 (ulcd.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 CHRIPS 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 obvious.
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 Oreotrichulus 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 Oreotrichulus, 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 Oreotrichulus leucopleurus from Argentina is sister to an O. estella individual from Peru, and both form the sister clade to the remaining species of Oreotrichulus. 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).

<table>
<thead>
<tr>
<th>Hummingbird clade stem age [Ma, 95% HPD]</th>
<th>Plant clade stem age [Ma, 95% HPD]</th>
<th>Ages distribution fitting?</th>
<th>Reference for plant clade age</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Basilinna</em> (Hylocharis) sauntii (2.75; 2.11–3.45/2.59; 1.78–3.41)</td>
<td><em>Arbutus peninsularis</em> (Eriaceae) (0.8; –)</td>
<td>No/No</td>
<td>Abrahamczyk &amp; Renner (2015)</td>
</tr>
<tr>
<td><em>Calypte</em> anna (2.17; 1.69–2.66/1.67; 1.3–2.22)</td>
<td><em>Ribles speciosum</em> (Grossulariaceae) (1.5; 0.14–3.27)</td>
<td>Yes/No</td>
<td>Abrahamczyk &amp; Renner (2015)</td>
</tr>
<tr>
<td><em>Eustoxeres</em> (21.45; 18.18–24.97/21.48; 19.44–23.7)</td>
<td><em>Centropogon granulosus</em> clade Campanulaceae; (a) (3.64; 2.27–10.1) (b) (2.26; 1.64–2.69)</td>
<td>No/No</td>
<td>(a) Antonelli (2009) (b) Lagomarsino et al. (2016)</td>
</tr>
<tr>
<td><em>Oreotrichulus</em> (6.8; –/7.78; 6.85–8.7)</td>
<td><em>Chuquiraga spinosa</em> clade Asteraceae; (10.81; 5.2–17.39 – Crown age: 7.1; –)</td>
<td>Yes/Yes</td>
<td>This study</td>
</tr>
</tbody>
</table>

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

|align*54x77| alistic hummingbirds by > 90%. The ranges of *Calypte anna*, *Basilinna* (*Hylocharis*) *xantusi* and the two species of *Sepha-

|align*54x77| noideae 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 *Eutox-

|align*54x77| eres* species as well as the *Chuquiraga spinosa* species group and the relevant *Oreotrichilus* species, the mutual range overl-

|align*54x77| laps were between 38% and 63% (Fig. 2, Tables 3 & 4). *Oreotrichilus* 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 *Oreotrichilus/Chuquiraga spi-

|align*54x77| nosa* species groups, we found matching ages of both part-

|align*54x77| ners, while for the *Eutoxeres/Centropogon granulosus*, *Basilinna xantusi*/Arbutus peninsularis and *Sephanoides/Tris-

|align*54x77| terix 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 hum-

|align*54x77| mingbird species with extremely curved bills and numerous species of *Chuquiraga spinosa* in Patagonia, and *Oreotrichilus* 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 *Oreotr-

|align*54x77| ichilus/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 *Sepha-

|align*54x77| noideae sephanoides* that do not co-occur with these plant spe-

|align*54x77| cies still migrate (Schuchmann, 1999).

In the *Oreotrichilus/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 cen-

|align*54x77| tral Andes 6–10 Ma ago (Gregory-Wodzicki, 2000). The *Chuquiraga spinosa* species group seems to have expanded into the high central and northern Andes from the southern temperate Andes (Excurra, 2002), while *Oreotrichilus* may have originated in the northern Andes as suggested by the distrib-

|align*54x77| ution of its closest relatives, *Polyonymus caroli* and *Opistho-

|align*54x77| prora euryptera*, which occur at elevation of 1500–3600 m (Schuchmann, 1999). *Oreotrichilus* may have begun coloniz-

|align*54x77| ing elevations up to 5200 m in co-evolution with the *Chuqi-

|align*54x77| raga 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 ances-

|align*54x77| tors of both clades diverged from their closest relatives, with the *Chuquiraga spinosa* species group then radiating from about 5.9 Ma onwards. *Oreotrichilus* 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 overl-

|align*54x77| lap 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–

|align*54x77| 10.1 Ma) ago. Species boundaries in the *Centropogon granulosus* clade, represented by 33 accessions in the study of Lago-

|align*54x77| marsino 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 pensinsularis, (c) Sephanoides/Tristerix aphyllus/corymbosus, (d) Eutoxeres/Centropogon granulosus clade and (e) Oreotrochilus/Chuquiraga spinosa clade.
The availability of a reliable food source is crucial for the evolution and distribution of birds. The corollas of Heliconia flowers were available when sicklebills evolved and that both species with the abruptly curved corollas depend on sicklebills for their gamete transport (see Fig. S1.1), both species have not yet been sequenced. 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. ampicorollinus 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.

**ACKNOWLEDGEMENTS**

We thank BirdLife International and NatureServe for providing distribution data of hummingbirds; the Cornell Museum of Vertebrates in Ithaca, NY, for providing tissues of Oreotrichilus leucopleurus; Tod Stuessy, Jon Rebman and Paul

---

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

<table>
<thead>
<tr>
<th>Mutualistic partners</th>
<th>Total area (1000 km²)</th>
<th>Area of co-existence (1000 km²)</th>
<th>Co-existence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calypte anna (resident) with Ribes speciosa</td>
<td>493.1</td>
<td>28.8</td>
<td>6</td>
</tr>
<tr>
<td>Calypte anna (non-breeding) with Ribes speciosa</td>
<td>777</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ribes speciosa with Calypte anna (resident)</td>
<td>28.8</td>
<td>28.8</td>
<td>100</td>
</tr>
<tr>
<td>Basilina (Hylocharis) xantusi with Arbutus pensularis</td>
<td>41.9</td>
<td>4.6</td>
<td>11</td>
</tr>
<tr>
<td>Arbutus pensularis with Basilina (Hylocharis) xantusi</td>
<td>4.6</td>
<td>4.6</td>
<td>100</td>
</tr>
<tr>
<td>Sephanoides spp. (resident-breeding) with Tristerix aphyllus/corymbosus</td>
<td>252.1</td>
<td>119.3</td>
<td>47</td>
</tr>
<tr>
<td>Sephanoides spp. (non-breeding) with Tristerix aphyllus/corymbosus</td>
<td>550.2</td>
<td>2.3</td>
<td>0</td>
</tr>
<tr>
<td>Sephanoides spp. (breeding) with Tristerix aphyllus/corymbosus</td>
<td>302.2</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>Tristerix aphyllus/corymbosus with Sephanoides spp. (resident)</td>
<td>132.4</td>
<td>121.7</td>
<td>92</td>
</tr>
<tr>
<td>Eutoxeres spp. with Centropogon granulosus species group</td>
<td>610.2</td>
<td>367.3</td>
<td>60</td>
</tr>
<tr>
<td>Centropogon granulosus species group with Eutoxeres spp.</td>
<td>664.5</td>
<td>367.3</td>
<td>55</td>
</tr>
<tr>
<td>Oreotrichilus spp. with Chuquiraga spinosa species group</td>
<td>1055</td>
<td>400.3</td>
<td>38</td>
</tr>
<tr>
<td>Chuquiraga spinosa species group with Oreotrichilus spp.</td>
<td>630.7</td>
<td>400.3</td>
<td>63</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Mutualistic partners</th>
<th>Total area (km²)</th>
<th>Area of co-existence (km²)</th>
<th>Co-existence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oreotrichilus spp.</td>
<td>1055</td>
<td>400.3</td>
<td>38</td>
</tr>
<tr>
<td>Oreotrichilus adela</td>
<td>50.6</td>
<td>19.7</td>
<td>39</td>
</tr>
<tr>
<td>Oreotrichilus chimborazo</td>
<td>36.9</td>
<td>32.1</td>
<td>87</td>
</tr>
<tr>
<td>Oreotrichilus estella</td>
<td>595.8</td>
<td>263.1</td>
<td>44</td>
</tr>
<tr>
<td>Oreotrichilus leucopleurus</td>
<td>404.1</td>
<td>80.9</td>
<td>20</td>
</tr>
<tr>
<td>Oreotrichilus melanogaster</td>
<td>55.3</td>
<td>43.5</td>
<td>79</td>
</tr>
<tr>
<td>Oreotrichilus stolzmanni</td>
<td>61.4</td>
<td>55.8</td>
<td>91</td>
</tr>
</tbody>
</table>
S. Abrahamczyk et al.

Sorensen for helpful comments; the herbaria of Munich (M, MSB), Missouri Botanical Garden (MO), New York (NY), Stockholm (S), and Vienna (WU) for providing material for sequencing; Joseph C. Boone for providing the photo of Oreotrochilus chimborazo; Martina Silber, Gülperi Stenhousen and Daniel Mendes for support in the laboratory; and three anonymous referees, the handling editor, Şerban Proches, and the editor in chief Peter Linder, for helpful comments.

REFERENCES


**SUPPORTING INFORMATION**

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

**Appendix S1** Supplementary figures and tables.

**BIOSKETCHES**

**Stefan Abrahamczyk** is a postdoc at the University of Bonn working mainly on the ecology and evolution of plant–animal interactions and plant mating systems.

**Susanne Renner** is a Professor of Plant Systematics and Evolution at the University of Munich.

**Constantin Poretchkin** is a PhD student at the University of Bonn working on the biodiversity of Andean plants.

Editor: Şerban Procheș