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Biodiversity cradles and museums segregating within hotspots of endemism

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The immense concentrations of vertebrate species in tropical mountains remain a prominent but unexplained pattern in biogeography. A long-standing hypothesis suggests that montane biodiversity hotspots result from endemic species aggregating within ecologically stable localities. Here, the persistence of ancient lineages coincides with frequent speciation events, making such areas both 'cradles' (where new species arise) and 'museums' (where old species survive). Although this hypothesis refers to processes operating at the scale of valleys, it remains supported primarily by patterns generated from coarse-scale distribution data. Using high-resolution occurrence and phylogenetic data on Andean hummingbirds, we find that old and young endemic species are not spatially aggregated. The young endemic species tend to have non-overlapping distributions scattered along the Andean treeline, a long and narrow habitat where populations easily become fragmented. By contrast, the old endemic species have more aggregated distributions, but mainly within pockets of cloud forests at lower elevations than the young endemic species. These findings contradict the premise that biogeographical cradles and museums should overlap in valley systems where pockets of stable climate persist through periods of climate change. Instead, Andean biodiversity hotspots may derive from large-scale fluctuating climate complexity in conjunction with local-scale variability in available area and habitat connectivity.

1. Introduction

The majority of Earth's vertebrate diversity concentrates within tropical mountains, with the Andes standing out as the most species-rich region [1–5]. The overall high diversity of the Andes derives from numerous local hotspots with immense concentrations of endemic (small-ranging) species [6–8]. A classic explanation is that high speciation rates combine with the long-term persistence of local populations [6], making the endemism hotspots both cradles for young (recently derived) species and museums for old (early-divergent) species [6,9–12]. The premise of this explanation is that the expected species distribution patterns are conserved at the scale of valleys where the relevant speciation-extinction processes occur [1,6,10,13–16]. Analyses of broad-scale patterns in species distributions confirmed that young and old lineages tend to aggregate within tropical mountains [9–12,17,18]. Moreover, recent advances in process-based models revealed that the tropical Andes are optimal for speciation and species persistence through recurring periods of climate fluctuations [19]. However, previous investigations typically used species

distributions mapped at coarse spatial resolutions, as those are the data available at continental and global scales [9–11,17,18,20,21]. Although the patterns obtained at coarse spatial resolutions may statistically accord with the hypothesis's predictions, the results may not scale down to valley systems where the hypothesized speciation–extinction dynamics operate [6]. These processes can operate on either side of a deep valley, such as the Marañon valley [22], or between small adjacent valleys such as those found in the Colombian east Andes [16]. Thus, valley systems provide a suitable reference scale for gaining new insight into the anticipated aggregation of young and old endemic species.

The role of the Andes as a biodiversity cradle has been linked to the fluctuating climates of the Quaternary [23–25]. Topographic complexity combined with climate fluctuations over time (termed *fluctuating climatic complexity* [19]) may have caused cyclical phases of connectivity and fragmentation of highland habitats [26,27], causing local species populations to become isolated and eventually diverge in allopatry [9,27–29]. In addition to Quaternary climate fluctuations, high rates of speciation in Andean forests may result from stochastic events. Graves [13,14] noted the phenotypic disparity among congeneric birds peaks in elevational zones around the treeline. In this narrow and elongated habitat band, demographic and environmental stochasticity may cause the fragmentation of local populations [13,14]. Accordingly, previous studies find the highest speciation rates in the Andean highlands [16,23,24,28]. Demographic fluctuations in population sizes or extreme weather events can also wipe out small and isolated populations, implicating that stochastic mechanisms may also result in extinction. In his classical work, Fjeldså [6] proposed that the persistence of new species depends on habitats with locally stable climates. Such stable climates occur at the scale of valleys where cloud forests flank warm intermountain basins, or atmospheric inversions create permanent mist zones that maintain humidity [7]. As such, Andean hotpots of endemism are hypothesized to form within so-called 'extremely ecologically stable areas' [6] where the distribution of old and young endemic species overlap [6,9,10].

We investigate this now-classic hypothesis using all Andean hummingbirds ($n = 229$ species) as a model taxon representing a species-rich mountain clade. Our analytical approach is not focused on generalized correlations with environmental data. That is because current data on historical climate stability over the past 100 000 yrs to 1 Myr is at a coarse spatial resolution compared to the scale of valleys where the hypothesized processes operate. Acknowledging the historical climate data's limitations in scale of resolution, we instead tested the hypothesis's underlying premise, i.e. the pattern where hotspots of young endemic species are spatially congruent with hotspots of old endemic species. Hummingbirds (Trochilidae) are suitable for testing this hypothesis for several reasons: (i) their taxonomy is well resolved [30]; (ii) their geographical ranges are well known; (iii) their systematic relationships are supported by a phylogeny comprising the majority of species [31]; and (iv) hummingbirds reach their greatest diversity in the Andes region, representing the number one hotspot for birds in the world [1]. We use high-resolution distributions at the 0.25° scale (see Methods). We defined a hummingbird within the first range size quartile as 'endemic' ($n = 58$; figure 1*a*; [1,33]), which corresponds to a geographical range size of less than 97 000 km^2. We then use the phylogeny

to divide endemic species into two age classes: young species (the 25% fraction of endemic species with the shortest terminal branches; $n = 15$) and old species (the 25% fraction of endemic species with the longest terminal branches; $n = 15$; figure 1*b*,*c*). Using this quartile method allows us to map the geographical overlap between specific subsets of the total species assemblage (i.e. young versus old endemic species), as is required for testing Fjeldså's classic hypothesis [6].

2. Results and discussion

Contrary to *a priori* expectations, we found that young and old endemic species were geographically segregated—not aggregated (figure 2; $\chi^2_3 = 156.53$, $p < 0.001$; electronic supplementary material, table S1a and figure S1b). Several old endemic species aggregated in the northern Andes, whereas the young endemic species dominated the Santa Marta Mountains in Colombia and Cordillera Carpish in central Peru. In the remaining parts of the study region, young and old endemic species had segregated distributions, although within close geographical proximity of each other. The only localities where we found a high standardized richness of both young and old endemic species were in the Colombian east Andes and the Cuzco region of Peru. Both areas have complex topographies with deep valleys intersecting tall mountain ridges within close proximity. Hence, the landscape is a mosaic of different habitat types, causing overlap between young and old endemic species. Notably, the east Colombian Andes proved suitable for studying speciation processes under fluctuating climate complexity [16,27].

To quantify the range overlap between the two age classes, we applied a biogeographical null model that standardized endemic species richness by a grid cell's total species richness (see Methods). These analyses revealed that young and old endemic species contributed differently to local diversity hotspots. We found that young endemic species had scattered distributions with low range overlap, whereas old endemic species occurred in aggregated hotspots with higher range overlap (figure 3). At the 0.25° scale, the richness of young endemic species rarely exceeded two, whereas old endemic species were represented in grid cells with up to five species. This result indicates that local hotspots of endemism consist mostly of old species. Moreover, the high replacement of young endemic species throughout the mountain range could suggest that the recently derived species have still not expanded their ranges into secondary contact zones. This pattern is well illustrated in the genus *Metallura*, where five young endemic species distribute along the Andean Cordilleras with little or no geographical overlap ([24]; electronic supplementary material, figure S2). A similar vicariant mode of speciation is also found in other bird groups, including spinetails *Cranioleuca*, brush finches *Atlapetes*, canasteros *Asthenes*, flowerpiercers *Diglossa*, and tapaculos *Scytalopus* [13,14,34]. Whether species in these groups will maintain their segregated distributions over time remains an open question. Under a neutral model of range dynamics, recently derived sister species should eventually transition to secondary sympatry [35], but the rate may depend on several different processes. Among Furnariidae birds in South America, the transition to secondary sympatry following a speciation event is initially slow, which coincides with expectations of interspecific competition limiting range
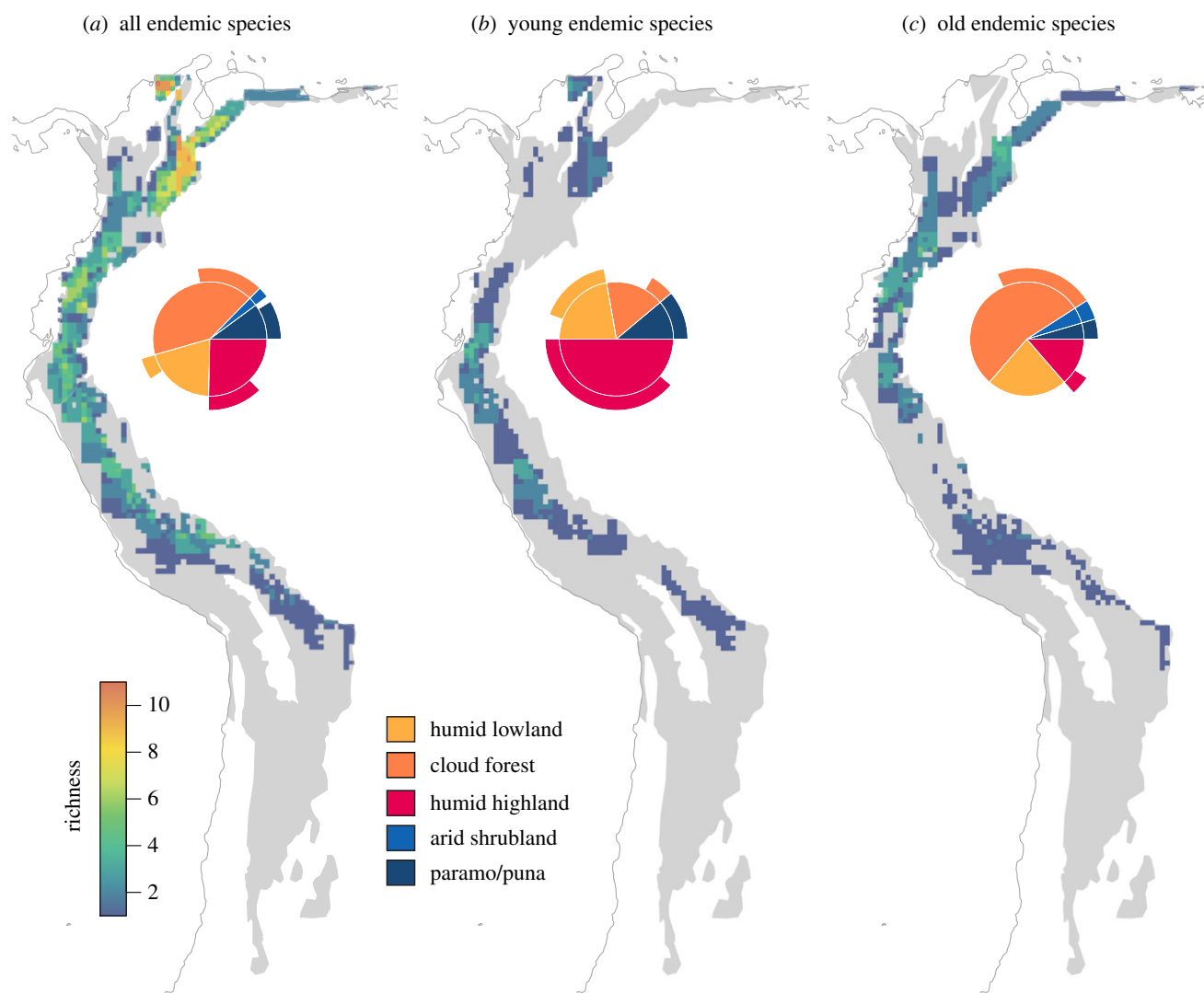
Figure 1. Regional richness pattern of young and old endemic species. (*a*) The richness of endemic hummingbirds is defined as the 25% fraction of species in the north and central Andes (grey shade; [32]) with the smallest geographical ranges. (*b*) The richness of young endemic species, i.e. the 25% fraction of endemic hummingbirds with the shortest terminal branch lengths. (*c*) The richness of old endemic species, i.e. the 25% fraction of endemic hummingbirds with the longest terminal branch lengths. The pie diagrams show the differences in the species' habitat preferences. The inner circles represent the frequency distribution of hummingbird species occurring in one or several habitats. The outer diagram shows the fractions of hummingbirds that are restricted to one single habitat. (Online version in colour.)

overlap between recently derived lineages [36]. Alternatively, a slow initial rate of secondary sympatry could reflect young highland species dispersing vertically to lower elevations, instead of horizontally [37], following the increased availability of surface area and habitat connectivity downslope [13,14,37], or cyclic phases of glaciation and inter-glaciation periods [16,27].

The overall dynamic environment characterizing Andean highlands is probably unsuitable for maintaining old endemic species. Whether these species' ranges have always been small or have contracted into relictual patches remains an open question. The old endemic species' tendency to aggregate may reflect historical range contractions into refugia [37,38]. In accordance with this idea, we found that young and old and endemic species had distinct habitat preferences (figure 1; Fisher's exact, $p = 0.026$; electronic supplementary material, table S3). Old endemic species are generally found at lower elevations than young endemic species (figure 4*a*) and mostly in the lower parts of the montane evergreen forest (cloud forest zone; figure 1). By contrast, young endemic species associate more with humid highland

vegetations, such as the mosaics of stunted elfin forests (figure 1). Their distributions peak slightly above 3000 m, approximating the Andean treeline ecotone (figure 4*a*). The elevational distributions of young and old endemic species were similar when using different approaches to adding missing species to the phylogeny (electronic supplementary material, figure S10f). Moreover, their distributions did not merely reflect a mid-domain effect (as a stand-alone hypothesis): the young endemic species are more species-rich at approximately 3000 m than what the mid-domain null model predicts. Vice versa, the old endemic species are more species-rich at approximately 1500 m than expected (electronic supplementary material, figure S7). Nevertheless, the mid-domain effect could still influence the asymmetric richness-elevation patterns of species richness in interaction with other factors such as contemporary and historical changes in environmental conditions [39]. In our data, the habitat mosaic of the treeline ecotone appears to aggregate young endemic species, whereas the cloud forest aggregates old endemic species at lower elevations. As additional support for this pattern, we find that the average terminal branch lengths
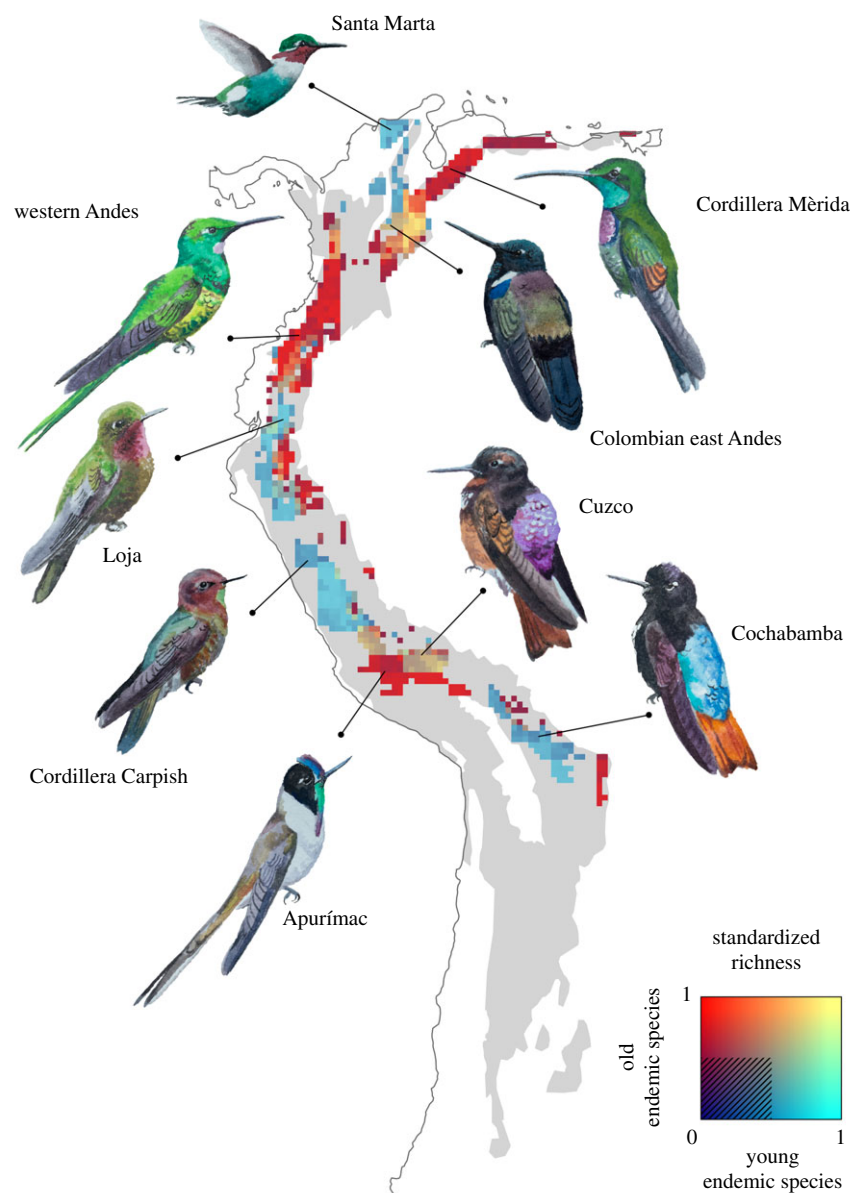
Figure 2. Disjunct distributions of young and old endemic species. Colours highlight grid cells with more young or old endemic species than expected from a biogeographical null model. The grey-shaded area marks the central and northern Andes [32], constituting the biogeographical source pool. The paintings (by Jon Fjeldså) show representatives of young and old endemic species from different regions in the Andes. (Online version in colour.)

for all endemic species vary significantly across elevations (figure 4b), where species at mid-elevations (1500–2000 m) had the longest terminal branch lengths (Kruskal–Wallis $\chi^2_7 =$ 15.07, $p = 0.032$). This pattern in terminal branch lengths shows that the elevational segregation of young and old endemic species is also visible at the species assemblage level.

Our results support previous suggestions that the patchy treeline ecotone is a centre for recent speciation in the Andes [13]. This idea appears analogous to previous work in Africa highlighting the distinct forest-savannah ecotones, which is a hotspot for new species [6,40–44]. The treeline zone comprises a small, fragmented surface area (electronic supplementary material, figure S4) that could stimulate population fragmentation. Small and fragmented populations are susceptible to demographic and environmental stochasticity; hence this environment may provide few opportunities for maintaining old endemic species [19,24]. The cloud forest at lower elevations covers a broad range of elevations (electronic supplementary material, figure S5) and thus may have retained high connectivity during the

Quaternary [45]. Accordingly, species at mid-elevation tend to have broader elevational range limits than species in the lowlands and highlands (electronic supplementary material, figure S6). Moreover, the cloud forest is ecologically distinct from lowland habitats because it has a cooler climate and a stable condensation zone [10]. We find that lowland species typically have narrower elevational range limits but larger geographical ranges (electronic supplementary material, figure S6) following the geographical configuration of humid lowland vegetations [46]. Thus, while the elevational range limits are broadest around 2000 m, the geographical range size decreases systematically with elevation.

The combination of unique ecological conditions and habitat connectivity could explain why the Andean cloud forests preserve endemic phylogenetically early-divergent species (i.e. the old species). This idea does not preclude that young endemic species should be absent from the lowlands entirely and that old species should be absent from the highlands. For instance, a young lowland species would not be expected to have the same elevational range as
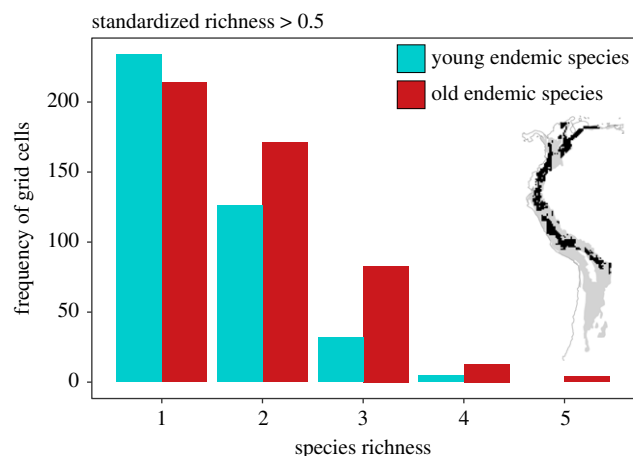
Figure 3. Richness-frequency distribution of young and old endemic species. The plot comprises the subset of grid cells with a standardized richness of young or old endemic species exceeding 0.5 (highlighted in black on the map). The threshold of 0.5 indicates that grid cells contain more of either age class than sampled by the majority of null model iterations. (Online version in colour.)
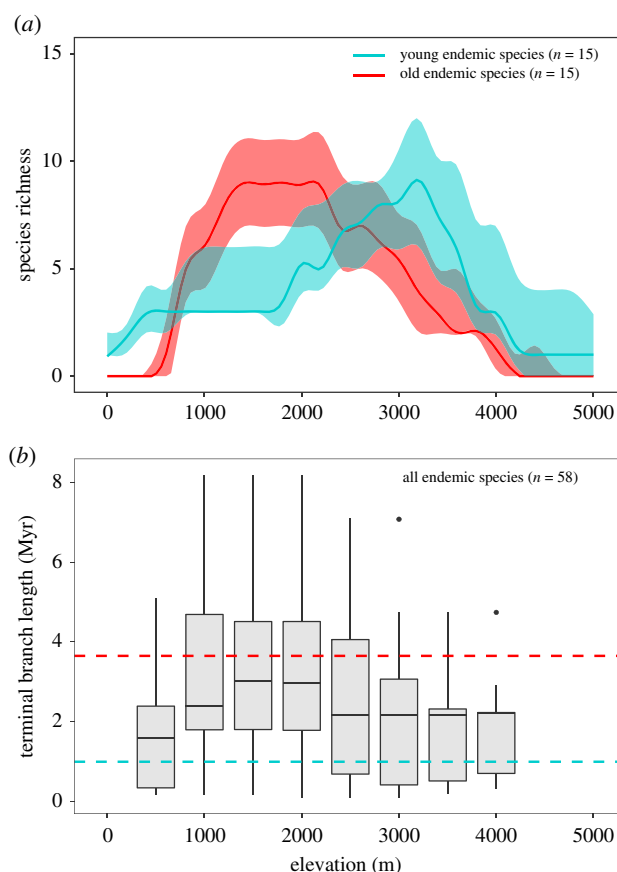

Figure 4. Segregations in elevations between young and old endemic species. (*a*) The richness of young endemic species peaks at higher elevations than old endemic species. Adding missing species to the phylogeny caused slight uncertainty in the composition of young and old endemic species (electronic supplementary material, table S10e). This uncertainty produces the figure's 95% confidence intervals (shaded areas). (*b*) Elevational variation in terminal branch lengths in million years (Myr). Here, missing species were added to the phylogeny using the median branch length for 1000 phylogenetic trees (electronic supplementary material, table S10a). The dashed lines represent the quartile boundaries used for selecting young (blue) and old (red) endemic species. (Online version in colour.)

young highland species. Accordingly, we find that species with narrow and wide elevational ranges have similar terminal branch lengths (electronic supplementary material, figure S8, panel A). However, our explanation for our observed patterns does predict that the broad cloud forest zone at mid-elevation should lack species with narrow elevational ranges, assuming the species range limits follow the altitudinal configuration of vegetation. That is also what we observed, namely, the fewest species with narrow elevational ranges at 1500–2000 m (electronic supplementary material, figure S8, panel B).

A potential caveat concerning our analysis is that young and old endemic species may cluster into different lineages in the phylogeny (electronic supplementary material, figure S11). However, we found that both classes of endemic species were broadly distributed in the phylogeny: neither young nor old endemic species were significantly more clustered in the phylogeny than random assemblies of endemic species (old endemic species, net relatedness index (NRI) = −0.32, $p = 0.31$; young endemic species, NRI = 1.84, $p = 0.06$). Thus, the geographical segregation of young and old endemic species appears not to have emerged from niche conservatism or borders separating biogeographical regions. Another caveat is the high proportion of endemic species added to the existing phylogeny (26.67% for young endemic species; 46.67% for old endemic species; electronic supplementary material, table S10c), as is a feature of most so-called complete phylogenies for larger clades. Thus, the branch length of each added species has an uncertainty, which introduces minor variations in the age classes' species composition (electronic supplementary material, table S10e). This variation in species composition causes the confidence intervals in figure 4a. Thus, the segregation between old and young endemic species is robust to the placement of unsampled species in the phylogeny. Thirdly, the division of endemic species into quartiles results in a small sample size that may limit statistical power ($n = 2 \times 15$). Henceforth, we investigated the elevational segregation of young and old lineages using the terminal branch length of all endemic species ($n = 58$; figure 4b). Regardless of the variability in sample size, the elevational patterns remain robust (figure 4).

Hummingbirds exemplify one of the many taxonomic groups that are highly species-rich in the Andes relative to the Amazonian lowlands. Therefore, the clade contributes to the general richness patterns that characterize the Andes as a global biodiversity hotspot [1,6,9,10,19,33,47]. The area's high concentration of endemic species has traditionally been explained by rapid allopatric speciation coinciding with low extinction rates through climate change periods [6,9,10,12]. The result becomes a local aggregation of endemic species with both early-divergent and recently derived origins [6,9,10]. Broad-scale geographical patterns support this idea [9,10,17,19], although a recent phylogenomic assessment on New World Suboscines challenged the view that tropical regions are hotspots for recent speciation [21]. The study found the highest speciation rates in temperate latitudes, leaving the tropics as a museum of primarily early-diverging lineages. The tendency for speciation to peak outside the tropics is also true for hummingbirds, given that the most recent and rapidly diverging clade (the so-called 'bees') represents the only successful invasion of North America [31]. However, such broad-scale evolutionary patterns struggle to capture speciation events over elevational gradients, where speciation processes operate at local spatial resolutions constrained by
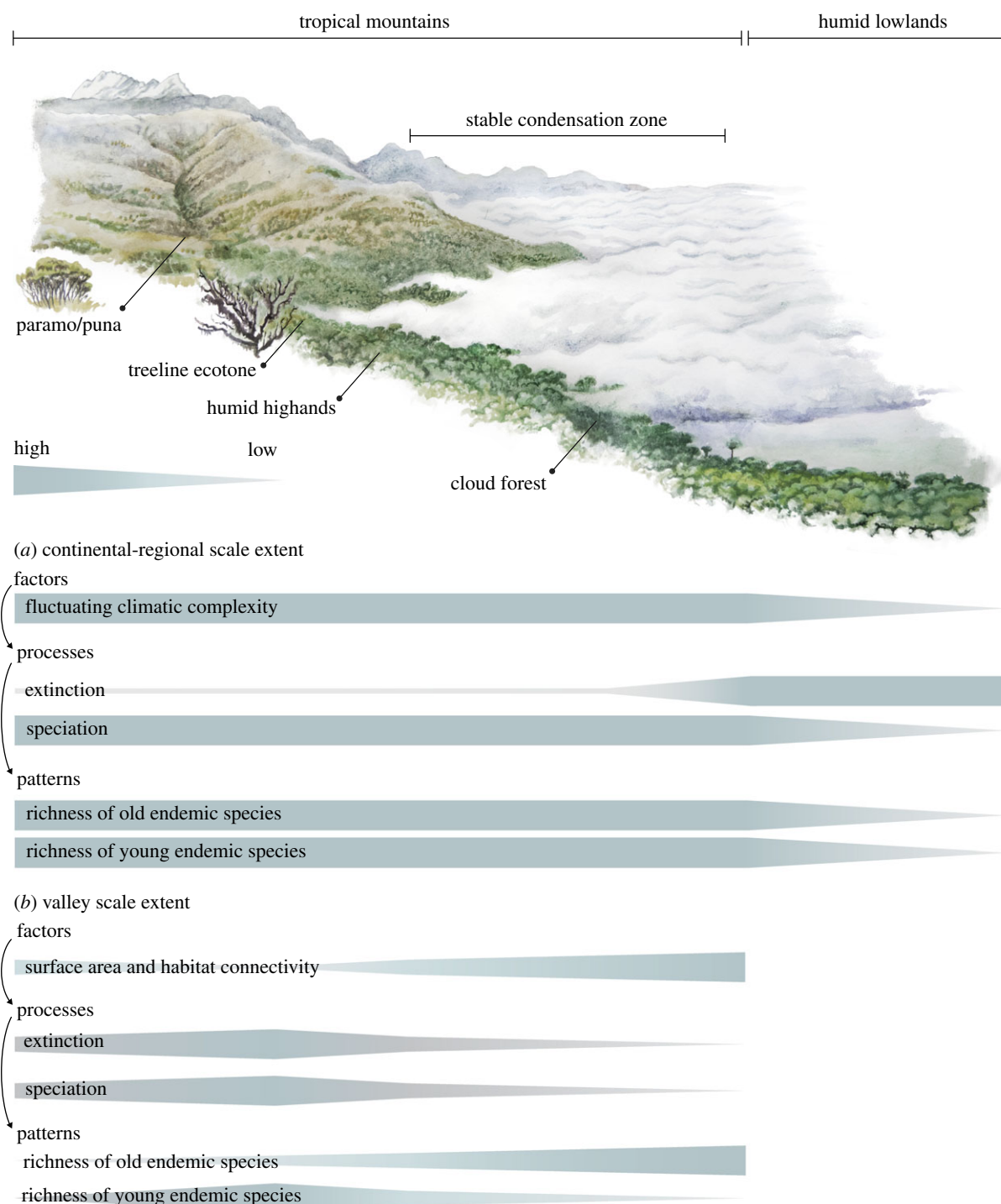
Figure 5. Hypothesized scale-dependency of speciation and extinction processes within a tropical mountain system, represented by the east Andean Cordillera (illustration by Jon Fjeldså). The grey bars' thickness indicates the levels of the factors, processes and patterns at different elevational zones. At the regional–continental scale of extent (a), historical climate dynamics in conjunction with topographic complexity (termed 'fluctuating climatic complexity'; [19]) promotes speciation and lowers extinction rates. The result is young and old endemic species aggregating in tropical mountains [9,10,19]. At the scale of valleys within mountains (b), surface area and habitat connectivity become more important determinants of the speciation and extinction processes. The narrow treeline zone with patchy vegetation promotes speciation at high altitudes, resulting in a scattered distribution of young endemic species [13,14]. Above the treeline ecotone, the surface area and habitat connectivity increase if the highlands reach a plateau. The habitats' width and connectivity increase towards the cloud forest at mid-elevation, providing more optimal conditions (i.e. low extinction) for the persistence of old endemic species. (Online version in colour.)

topography and local environmental conditions [6,13,14]. At this scale, the distribution of young endemic species suggests extensive allopatric speciation in Andean highlands (figures 3 and 4). However, local centres of speciation may be segregated from stable localities with low extinction rates when building up diversity at the regional–continental scale.

The idea of endemism hotspots forming within climatically stable areas builds on the premise that young and old endemic species overlap at local-landscape spatial resolutions [6]. Our results contradict this premise by showing that young and old endemic species segregate between valleys and elevational zones. Instead, our results coincide with fluctuating climate complexity, not climate stability, causing high speciation in conjunction with low extinction [19] (figure 5a). At the scale of valleys, these processes seem to operate in segregated locations, not because of climate differences but

owing to the variability in surface area and habitat connectivity (figure 5b). The wide-extending cloud forest zone rarely becomes fragmented under climate fluctuations and thereby constitutes stable habitats required for the long-term persistence of populations. By contrast, the narrow and patchy treeline ecotone contributes to high speciation, as populations easily become fragmented by climate fluctuations or stochastic events. This proposed idea unites classical literature on population connectivity [13,14] with the recent literature on fluctuating climate complexity [16,19,27]. Moreover, the idea explains the observed patterns of old and young endemic species and why their co-occurrence patterns are not spatial-scale invariant. It also explains why global biodiversity hotspots are often found in mountain regions and why mountain regions are extraordinarily species-rich, especially at the regional scale, compared with adjacent lowlands.

3. Material and methods

(a) Species distributions

The geographical distributions of hummingbirds were retrieved from an updated version of the bird distribution dataset mapped from primary-level sources at the scale of $1° × 1°$ grid cells ([30,48]; see Holt et al. [49] for methods and data sources). The extracted data comprised all extant hummingbird species that were found in the central and northern Andes ($n = 229$; using the International Ornithological Community taxonomy v. 10.1; [50]). The exact boundaries of this region were defined by the mountain polygons presented by Rahbek et al. [32]. From the literature, we also compiled information on the species' minimum and maximum elevations [51] (see the electronic supplementary material, table S9a for additional references). We combined the species' geographical distributions with their elevational range limits into range maps at the 2 arc-minute resolution [52]. A species was considered present in 2 arc-minute grid cells that overlapped both with its distribution at the $1° × 1°$ scale and its elevational range limit. One newly described species (Oreotrochilus cyanolaemus) was sampled with the same minimum and maximum elevation. To avoid over-filtering its distribution, we added a 50 m buffer on both sides of its elevational range. Finally, the species' distributions were aggregated to $0.25° × 0.25°$ resolution. The range size of each hummingbird was calculated as the number of occupied $0.25° × 0.25°$ grid cells, cf. Rahbek [53]. The process of clipping the species 1° ranges removed commission errors, reducing the range size by up to 77% (electronic supplementary material, table S9c). We then defined endemic species as those belonging to the first range size quartile (i.e. 25% of the species with the smallest global ranges; $n = 58$; [54–57]). Processing of the species' geographical ranges was done in R [58] using the geographical packages' raster' [59] and 'maptools' [60].

(b) Phylogenetic relationships

Estimates of the phylogenetic relationships among 189 species were retrieved from McGuire et al.'s time-calibrated tree [31]. The 26 of the 58 endemic species missing in the phylogeny were added based on prior taxonomic information (electronic supplementary material, table S10c). The remaining 14 species not included in the analysis remained unsampled in the phylogeny. We assigned branch lengths to the 26 added lineages (million years) based on the relatedness of sister species that were sampled in the phylogeny. For each sister species pair that was also represented by terminal branches in the phylogeny, we calculated the proportion of the branch length that separated

the two species from the stem leading to the speciation event (electronic supplementary material, table S10a). Subtracting this ratio from one gives the terminal branch length ratio (R), which is directly proportional to the terminal branch length of a missing species in the phylogeny. To determine the terminal branch length of an added species, we multiplied a randomly selected R-value with the terminal branch length of the sampled sister lineage. The procedure was repeated to generate 1000 trees, for which we listed the 25% fraction of the endemic species with the shortest terminal branches. The species most frequently falling into the first branch length quartile were identified as young (i.e. recently derived; $n = 15$; electronic supplementary material, table S10c). Similarly, we identified old endemic species (i.e. early divergent; $n = 15$) that most frequently felt into the fourth branch length quartile. To explore the consequences of different species compositions for our results, we ran sensitivity analyses using (i) a 'late-burst model' by adding species towards the tip of the sampled sister lineage, and (ii) an 'early burst model' by adding species towards the base of the sampled sister lineage (electronic supplementary material, table S10e). For this procedure, we repeated the existing protocol by subsetting R values to the 75% quantile (late-burst: $0.73 < R < 0.97$) and the 25% quantile (early-burst: $0.05 < R < 0.24$; in theelectronic supplementary material, table S10a). The processing of the phylogeny was done in the R [58] programming language using the packages' ape' [61] and 'phytools' [62].

Using quartiles to define young and old endemic species accords with previous analyses on the topic [1,10], while others preferred using phylogenetic summary statistics, such as phylogenetic endemism [17,63]. Phylogenetic endemism contains information about deep phylogenetic patterns for all species in an assemblage. By contrast, the classical hypothesis explains the distributions for a specific subset of species with the smallest geographical ranges, not average statistics of all species [6]. Moreover, the proposed effect of climate stability on the aggregation of young and old species operates in the phylogeny's periphery. Lineages may belong to an old phylogenetic branch while having a high recent speciation rate, as for Oreotrochilus and Oxypogon. Thus, we avoided phylogenetic summary statistics to obtain biogeographical patterns matching the original formulation in Fjeldså's hypothesis [6].

(c) Using biogeographical null models to identify biodiversity hotspots

The richness of endemic species is expected to be a function of total species richness. Thus, we applied a biogeographical null model to disentangle the pattern in endemic species from the underlying pattern of total richness. The null model compares the empirical richness of young and old endemic species to a random sample of all the species in the dataset. For a grid cell with species richness n, the algorithm randomly samples grid cells in a stepwise fashion, from which a species is randomly selected. The algorithm terminates when n unique species have been sampled. In this way, the null model preserves the total species richness while accounting for large-ranging species' potential for being sampled in more grid cells than small-ranging species. After running the null model 1000 times, we determined the standardized richness of young and old endemic species as the proportion of null model iterations that sampled fewer of a given age class than observed. The young and old endemic species were classified as over-represented in a grid cell when the majority of null model iterations sampled fewer of each respective age class than observed (standardized richness exceeding 0.5).

(d) Elevational analyses

We investigated the distribution of young and old endemic species along the elevational gradient using each species's minimum and

maximum elevational range limits. The analysis computes the richness of each age category in 10 m bands from 0 to 5000 m. Adding species to the phylogeny based on randomly sampled R-values caused slight uncertainty in the composition of young and old endemic species. Hence, we calculated the richness pattern for 1000 phylogenetic trees and extracted the 95% richness confidence interval. The same analysis was repeated for different approaches to adding missing species to the phylogeny: the 'early-burst' and the 'late-burst' models. We also investigated if the elevational pattern coincided with a mid-domain effect. We modelled the mid-domain effect by randomly shuffling young and old endemic species along the gradient while maintaining their elevational range coherency and using the empirical range frequency distribution following the guidelines by Colwell *et al.* [64]. After repeating the mid-domain model 1000 times, we compared the expected and empirical richness patterns. We used the 'ns' function from the 'splines' R package to present the results.

(e) Phylogenetic analyses

The evolutionary history of hummingbirds is characterized by persistently high diversification rates [31]. Old subfamilies (hermits and topazes) date back to the early Miocene (approx. 20 Ma), whereas the youngest subfamily (bees) diversified as late as the Pliocene (approx. 5 Ma). The vast age differences between subfamilies imply that the distribution of young and old endemic species could be explained purely by lineage-specific biogeographical histories, that is, hotspots of young endemic species consisting of recently derived subfamilies and old endemic species consisting of early-divergent subfamilies. Therefore, we tested whether young and old endemic species were associated with different lineages in the hummingbird phylogeny (electronic supplementary material, figure S11). Individually for young and old endemic species, we compared the mean pairwise distance in the phylogeny ($mpd_{observed}$) to a null model that randomly swaps age categories among the 58 endemic hummingbirds (mpd_{null}). $mpd_{observed}$ was calculated as an average across the populations of trees. mpd_{null} was calculated for a randomly selected tree in the population. After running the null model 1000 times, we calculated: (i) the NRI as ($mpd_{observed}$ − mean(mpd_{null}))/sd(mpd_{null}) [65]; and (ii) a p-value giving the proportion null models that sample a lower mpd_{null} than observed. The phylogenetic analyses were done in R [58] using the 'mpd' function from the 'picante' package [66].

(f) Hummingbird habitat preferences

Information on hummingbird habitat preferences was compiled by Stotz *et al.* [51]. From this compilation, we categorized five different habitats: arid shrubland (arid lowland shrub and arid montane shrub), paramo-puna grasslands, humid highland (elfin forest, *Polylepis* woodland and humid montane shrub), cloud forest (montane evergreen forest), and humid lowlands (comprising all remaining lowland habitats). We investigated whether hotspots of young and old endemic species were associated with different habitat types. The pie diagrams in figure 1 summarize the range of habitats occupied by all endemic species and the subset of young and old endemic species. Here, we applied Fisher's exact test to examine whether young endemic species differ from old endemic species in their habitat preferences.

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10

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