

# High Plant Diversity in Eocene South America: Evidence from Patagonia

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Tropical South America has the highest plant diversity of any region today, but this richness is usually characterized as a geologically recent development (Neogene or Pleistocene). From caldera-lake beds exposed at Laguna del Hunco in Patagonia, Argentina, paleolatitude  $\sim 47^\circ\text{S}$ , we report 102 leaf species. Radioisotopic and paleomagnetic analyses indicate that the flora was deposited 52 million years ago, the time of the early Eocene climatic optimum, when tropical plant taxa and warm, equable climates reached middle latitudes of both hemispheres. Adjusted for sample size, observed richness exceeds that of any other Eocene leaf flora, supporting an ancient history of high plant diversity in warm areas of South America.

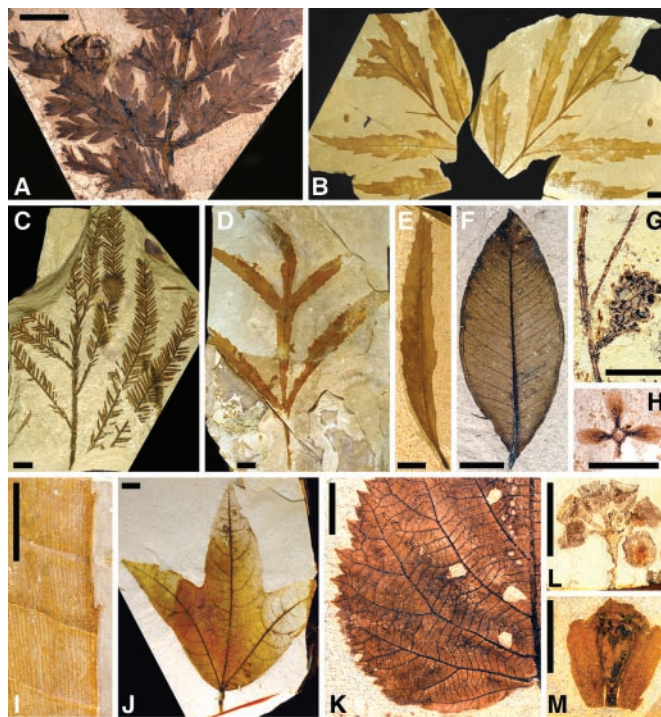
There is little evidence but much debate regarding how long the exceptional plant diversity of tropical South America has existed (1, 2). Most explanations have emphasized the late Neogene or Pleistocene (3–7), although the mechanisms and relative importance of geologically recent speciation are disputed (8–12). Evidence for or against earlier diversity is sparse (13–17). During the early Eocene, when maximum global temperatures for the Cenozoic occurred (18, 19), plants with tropical affinities grew at middle and high latitudes (20–23). From quantitative sampling of a middle-latitude flora, we present evidence for extraordinary plant diversity in early Eocene South America.

The flora we studied comes from the vicinity of Laguna del Hunco (LH) in northwestern Chubut Province, Patagonia, Argentina (24, 25). It is derived from tuffaceous caldera-lake deposits, known as the Tufolitas Laguna del Hunco, of the middle Chubut River volcanic-pyroclastic complex (25, 26). Previous K/Ar analyses of associated volcanic rocks have indicated a late Paleocene to middle Eocene age for the flora (27, 28). Marine sediments in nearby basins and tectonic evidence suggest that elevation was low and that the climate had a maritime influence (29, 30). The site is near the southern limit of the Paleogene Neotropical flora (20, 21, 23), and it

also contains a number of taxa that are extinct in South America today but have living relatives in Australasian floras (31–33) (Fig. 1).

We measured and correlated stratigraphic sections through the Tufolitas LH that contained

**Fig. 1.** Selected plant taxa representing the excellent preservation and taxonomic and morphological diversity of the Laguna del Hunco flora (33, 36). Scale bars, 1 cm. Parentheses indicate Museo Egidio Feruglio (MEF) specimen number and locality (Fig. 2). (A) Attached foliage of callitroid Cupressaceae similar to extant *Austrocedrus* (South America) and to several Australasian genera (MEF 971, loc. 13). (B) Attached compound leaf of "*Lomatia*" *preferuginea* (Proteaceae), part and counterpart, with lobed and toothed leaflets (972, 15). (C) Shoot and attached foliage of Podocarpaceae (973, 15). At least three other species of podocarps were found. (D) Complete, pinnatifid leaf of *Lomatia occidentalis* (Proteaceae) (974, float specimen). (E) "*Myrica*" *mira*, leaf (affinity unknown), with distinctive paired teeth along margin (975, 13). (F) Myrtaceae, leaf, showing prominent intramarginal vein (976, 13). (G) Attached infructescence and leaf of *Gymnostoma* sp. (Casuarinaceae, extant in Australasia). Note exerted bracts of infructescence and grooved surface of the nodular leaf (977, 22). (H) Propeller-like fruit, with four persistent sepals, of an unknown dicot species (?Cunoniaceae), with constricted sepal bases and thickened central disk (978, 6). (I) Cycad leaf similar to extant *Dioon*, with toothed margin (470, 4). (J) Malvales, three-lobed leaf, with basally actinodromous primary veins (979, 11). (K) Leaf-margin detail of unknown dicot species "TY62," showing compound, sharp-pointed teeth with flexuous or convex flanks and chevroned, opposite-percurrent tertiaries (980, 15). (L) Myrtaceae, infructescence (981, float specimen). (M) *Araucaria* sp. (Araucariaceae), attached seed and cone scale (982, 13). *Araucaria* foliage and a second type of cone scale were also found.



25 fossil localities, three datable tuffs, and six paleomagnetic reversals (Fig. 2) (34). We identified 1536 specimens of compression-impression plant macrofossils; nearly all (98%) were found between the 37- and 99-m levels of the aggregate systems (Fig. 2). Four quarries were selected for intensive sampling (64% of specimens) (Fig. 2).

Results from  $^{40}\text{Ar}/^{39}\text{Ar}$  analyses of the tuffs indicated ages near 52 Ma (million years ago) (Fig. 2) (34). The two youngest ages are at odds with superposition, but their confidence intervals either overlap or nearly overlap each other's means. From paleomagnetic results, we assign the most fossiliferous strata to the upper portion of magnetic polarity subchron (C) 23n.2r and the lower half of C23n.2n (Fig. 2) (34). These data place the flora within the early Eocene climatic optimum (EECO), an  $\sim 2$ -million-year interval that is known for the warmest sustained temperatures of the Cenozoic (19). At 52 Ma, the latitude of LH was  $\sim 47^\circ$  to  $48^\circ\text{S}$  (35).

In the flora, we recognize 102 leaf species (includes described species and undescribed morphospecies) of dicots, monocots, conifers, ginkgophytes, cycads, and ferns and an addi-

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tional 22 reproductive species from these groups (Fig. 1) (33, 34, 36). Dicots were the most diverse group, with 88 leaf species.

To evaluate species diversity relative to sample size, we derived rarefaction curves from relative abundance data of dicot leaves for the four most heavily sampled quarries, both individually (Fig. 3A) and in combination (Fig. 3B), as well as for the bulk flora (Fig. 3B). For comparison, we rarefied leaf counts for six Eocene floras from lacustrine and fluvial settings at middle paleolatitudes of western North America (Fig. 3). These six are diverse, well studied, and quantitatively sampled in a manner similar to the sampling at LH. The total known diversity of some of the North American floras is much greater

than indicated by rarefaction analyses, but this reflects selective sampling of unknown numbers of specimens over years or decades (37–39).

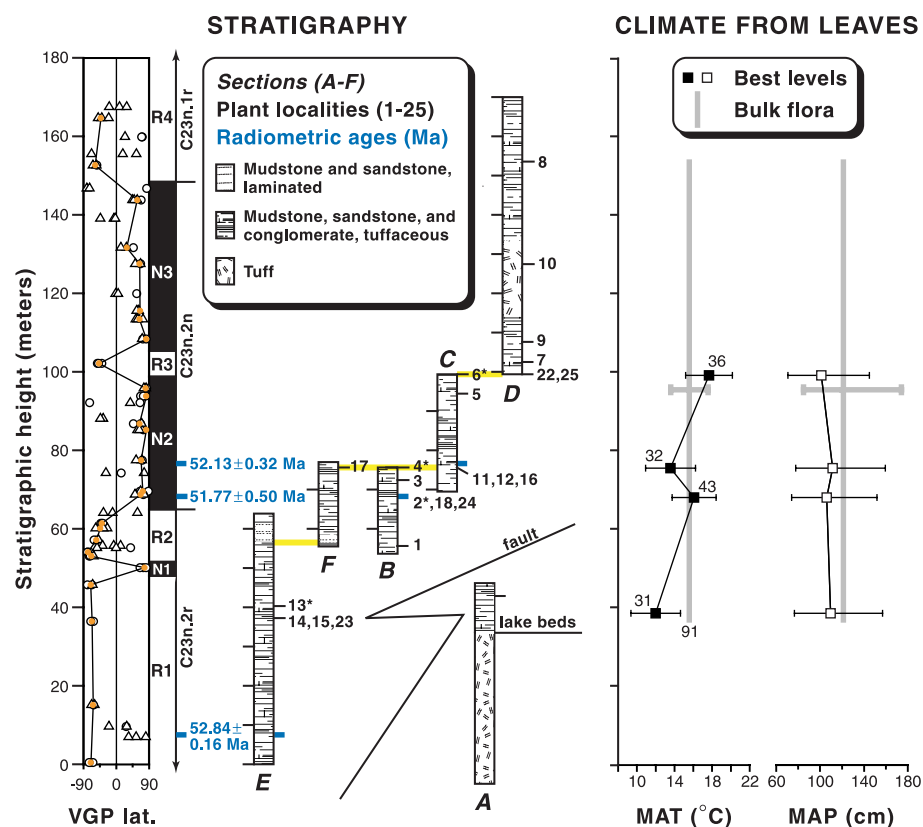
None of the North American floras provides a precise temporal and depositional analog to the LH flora, but they represent similar age (Wyoming floras), topographic and depositional setting (Republic, Green River, Florissant), or maritime climate (Puget Group, Chalk Bluffs) (legend to Fig. 3). The Green River and Florissant floras are known for preservation of attached plant organs (37). No Eocene North American floras are available from caldera lakes, a setting that might favor the preservation of plant diversity because of steep surrounding topography

(38, 40). However, topography surrounding the caldera lake at LH was more subdued than it was to the south (25), and even in lake basins with high relief, remains of plants from elevated areas are rare (41). The most reliable comparisons are those with single quarries from fossil lakes (Fig. 3A, Republic, Green River, and Florissant). The combined quarries (Fig. 3B) introduce varying amounts of temporal and spatial mixing that may increase diversity artifactually, with perhaps the greatest effect in the Puget Group (42).

The rarefaction analyses show that the LH flora is significantly more rich for its sample size than any Eocene leaf flora from North America (Fig. 3). Three of the four principal quarries from LH plot above (quarry 2) or within the 95% confidence limits of the most diverse North American localities (Fig. 3A). The same high diversity is apparent in rarefactions of the bulk flora and for subsets of the principal quarries (Fig. 3B). Nearly the same rarefaction curves result if the most diverse LH locality is removed (not shown). Thus, the elevated diversity observed at LH does not depend on a single locality or on the aggregation of numerous localities. Total richness also significantly exceeds that known from Eocene leaf floras of Germany (43), Australia (44), and Tanzania (45).

The mean annual temperature (MAT) estimated from leaf-margin analysis (34, 46) of the bulk flora is  $15.6 \pm 2.0^\circ\text{C}$  (Fig. 2). Individual sampling levels suggest an overall warming of  $\sim 6^\circ\text{C}$  (from  $\sim 12^\circ$  to  $18^\circ\text{C}$ ), although we place the greatest confidence in the bulk estimate because of the large number of species used (Fig. 2). Mean annual precipitation (MAP) is estimated from leaf-area analysis (47) as 100 to 120 cm, with no evidence of significant change within the sampled interval (Fig. 2) (34). This estimate should be taken as a minimum: The high diversity of the flora suggests that the upper ranges of leaf size for many rare species are not yet sampled, and transport into lakes generally selects against large leaves (48). The combined presence of palms, cycads, araucarian conifers, diverse podocarps, and *Gymnostoma* (Fig. 1), along with the absence of *Nothofagus*, provide evidence of an equable climate, with winter temperatures warmer than  $\sim 10^\circ\text{C}$  and abundant rainfall (20, 22, 32, 49–51).

The precipitation proxies indicate that the Patagonian Andes to the west of LH did not cast a significant rain shadow, supporting other evidence for their low elevation (29, 52). Our temperature data are corroborated by estimated sea-surface temperatures of  $16^\circ$  to  $17^\circ\text{C}$  during the EECO at four deep-sea sites from similar paleolatitudes in the South Atlantic (53). Marine and terrestrial proxy data from the Antarctic and from areas north of LH indicate temperatures that bracket our results latitudinally (53–55). Our estimated paleotemperatures for LH are



**Fig. 2.** Stratigraphic section of the Tufolitas Laguna del Hunco, aggregate thickness 170 m, showing principal lithologies; six local sections; correlations (yellow) along marker beds for the five continuous sections (B to F); plant localities; radiometrically dated samples, with 95% confidence intervals; virtual geomagnetic pole latitudes (VGP lat.); intervals of reversed and normal polarity (R1, N1, etc.), assignments to magnetic polarity subchrons (we interpret the single-site reversals, N1 and R3, as unidentified cryptochrons of short duration); and climatic results from leaf-margin (MAT) and leaf-area (MAP) analyses (34). The base of the Tufolitas LH was found only in section A, which could not be traced accurately across a fault to continuous sections B to F; these sections were measured on outcrops extending 1.3 km along a single drainage and were correlated by bed tracing. Section A is placed at an artificially high position in the figure (34). Most plant fossils occurred in tuffaceous mudrocks (34). Asterisks, the four principal quarries (see text and Fig. 3A). Red circles with connecting line, means of three individually measured, oriented paleomagnetic samples per site for which circular standard deviation was  $<35^\circ$ . Open circles, paleomagnetic sample means calculated by principal components analysis (59). Open triangles, sample means calculated by Fisher statistics (60). Labels show the number of species used in the estimates for both MAT and MAP. MAT error bars indicate  $\pm 1\sigma$  of binomial error or  $\pm 2^\circ\text{C}$ , whichever is greater (46); MAP error bars are  $\pm 1\sigma$  (47). Climatic results for the “best levels” include species from principal quarries and ancillary quarries excavated along strike (34). Bulk estimates include four species found only in float rocks not assignable to a precise stratigraphic level (34). Plant locality 19, not in a measurable position, is not shown.

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less than or approximately equal to most of the North American sites, and precipitation estimates are also mostly comparable (legend to Fig. 3). Thus, climate biases against or is neutral with regard to our observation of relatively high species richness at LH (56, 57).

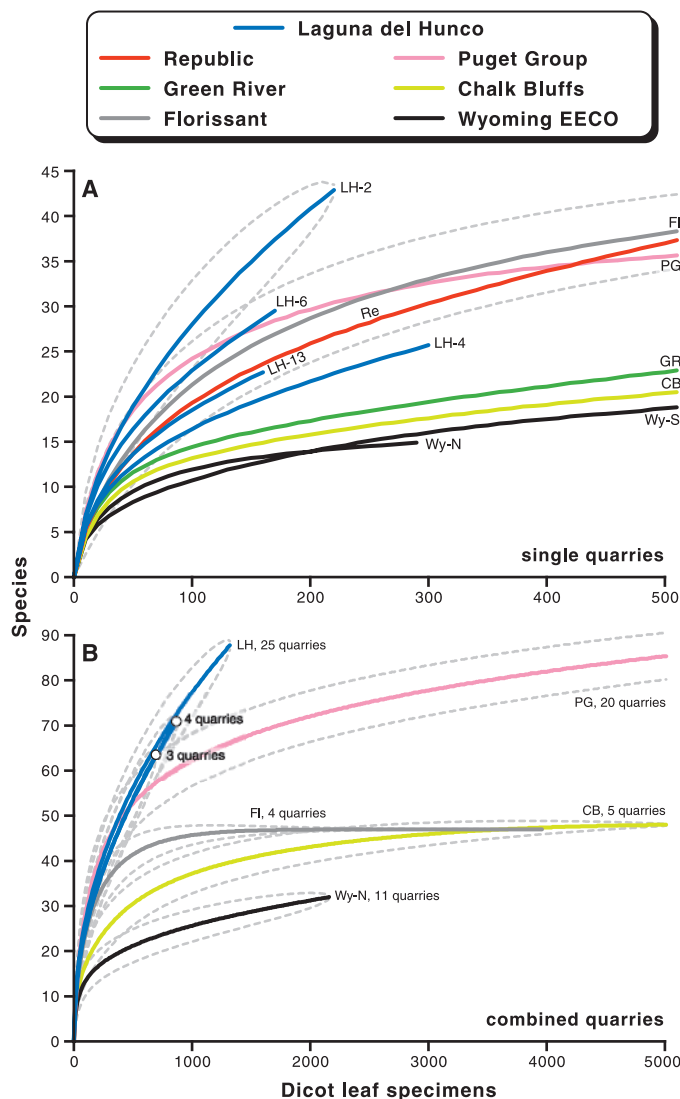
Other evidence also is consistent with elevated floral diversity in Paleogene South America. The Eocene flora of Río Pichileufú, from ~160 km NNW of LH, contains many of the same species as the LH flora and appears to be as diverse (33, 58), which suggests that rich, subtropical vegetation ex-

isted over a large portion of Eocene Patagonia. Palynological data from the Paleocene and Eocene of Colombia and Venezuela show significant diversification in association with warming temperatures and increased rainfall across the Paleocene-Eocene boundary, which suggests in situ speciation (16, 17). Finally, numerous plant families that are now speciose in South America have Paleocene and Eocene fossil records there (13, 15, 21), demonstrating persistence and suggesting early diversification.

The current richness of South American floras has resulted from many factors, which

include immigration, isolation, low extinction rates, and natural selection related to climate change and orogeny. These have been used to hypothesize a late Cenozoic origin of high Neotropical diversity, but our results suggest that elevated plant diversity is an ancient feature of South America.

**Fig. 3.** Rarefied richness of dicot leaf species at Laguna del Hunco and quantitatively sampled sites from the Eocene of North America. Left column in key and LH flora are lacustrine assemblages; right column is fluviially deposited floras. Dashed gray lines, 95% confidence intervals. Leaf-count data (33): Republic (61); Green River (62); Florissant (63); Puget Group (42, 64); Chalk Bluffs (65); Wyoming EECO (66, 67). (A) Single-quarry collections, labeled with abbreviations corresponding to the key, shown up to 500 specimens for detail and including all four principal localities for Laguna del Hunco (Fig. 2, asterisks); Wy-N and Wy-S label samples from northern (66) and southern (67) Wyoming, respectively. For North American floras with data from more than one locality, only the most diverse quarry is shown, and confidence intervals are given, for readability, only for the most diverse samples from LH and North America (Florissant). (B) Lumped counts of three or more quarries from a single area, as labeled and shown up to 5000



specimens. For LH, rarefactions are shown for all 25 quarries, for the four principal quarries as in (A), and for the three uppermost principal quarries (LH-2, 4, and 6). Rarefactions computed using Analytic Rarefaction 1.3, by S. Holland (68). Ages, geologic settings, and estimated MATs and MAPs (if available) from leaf-margin and leaf-area analysis; climatic estimates are adjusted from published values in some cases so that all MATs and MAPs in this paper are derived using the identical formulae (34): Republic, Washington, 49 to 50 Ma lake in volcanic highlands, ~13°C (61, 69); Green River flora (sample from Bonanza, Utah), ~43 to 48 Ma intermontane lake, ~15°C, ~84 cm (47, 70–72); Florissant, Colorado, ~34 Ma montane lake, ~12° to 13°C (37, 63, 73, 74); Puget Group flora, Washington, middle to late Eocene delta plain, ~16°C (42); Chalk Bluffs, California, 49 to 50 Ma fluvial system, ~17°C, ~160 cm (47, 65, 72, 75); Wyoming EECO, ~53 Ma swamps and distal plains, ~21° to 22°C, ~140 cm (18, 66, 67).

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- inspection of type material from the Eocene Río Pichileufú flora (58) confirms the reported taxonomic similarity to the LH flora (26), and our preliminary field data from Río Pichileufú suggest a diversity comparable to LH. Formal taxonomic knowledge of the LH flora is not yet sufficient to allow rigorous analysis of familial and generic diversity (34, 36). All 1215 voucher specimens for this study are housed at MEF, including exemplar specimens of each species. Fieldwork reported here took place in November 1999. We do not attempt to integrate our data with previous collections in the United States and Argentina, which would be inappropriate for our stratigraphic methodology.
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**Supporting Online Material**  
[www.sciencemag.org/cgi/content/full/300/5616/122/DC1](http://www.sciencemag.org/cgi/content/full/300/5616/122/DC1)  
 Materials and Methods  
 Figs. S1 to S6  
 Tables S1 to S7  
 References and Notes

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## Carotenoid Modulation of Immune Function and Sexual Attractiveness in Zebra Finches

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One hypothesis for why females in many animal species frequently prefer to mate with the most elaborately ornamented males predicts that availability of carotenoid pigments is a potentially limiting factor for both ornament expression and immune function. An implicit assumption of this hypothesis is that males that can afford to produce more elaborate carotenoid-dependent displays must be healthier individuals with superior immunocompetence. However, whether variation in circulating carotenoid levels causes variation in both immune function and sexual attractiveness has not been determined in any species. In this study, we show that manipulation of dietary carotenoid supply invokes parallel changes in cell-mediated immune function and sexual attractiveness in male zebra finches (*Taeniopygia guttata*).

Females in many animal species frequently prefer to mate with the most elaborately ornamented males (1), but how such displays reveal a male's worth is a contentious issue (2–5). For a signal to honestly reveal an individual's quality, it must be costly to

produce (6, 7). The expression of many ornamental traits depends on carotenoids, red and yellow pigments that animals cannot synthesize de novo and ultimately must obtain through their diet (2–5). Carotenoids are antioxidants and immunostimulants (8, 9), and it has recently been hypothesized that a trade-off exists in carotenoid allocation between maintaining health and ornamentation: Males in better condition should require fewer carotenoids for immune function and could therefore use more of this resource to enhance ornamental display, thereby advertising their superior health (10, 11). However, whether variation in

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2006. The number of grains will be limited (~100 interstellar and ~1000 cometary grains), but but determination of cosmic ray exposure ages of interstellar dust, cometary GEMS/IDPs, and crystalline silicates will be very revealing.

Techniques are also being developed to identify, in samples collected in Earth orbit, those grains that are true interstellar dust grains among IDPs that may have been processed in the solar system (14). Collectors in Earth orbit using such techniques would provide a relatively cheap way to collect large samples of “fresh” interstellar dust. Moreover, this would yield IDPs that have not been exposed and al-

tered by the heating events associated with their entry into Earth’s atmosphere.

Studies of captured IDPs and “interstellar” dust will help to address the issue of the presolar nature of GEMS and the origin of comets and will be an important step toward putting the planetary formation jigsaw puzzle together on a “grain-by-grain” basis.

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## ECOLOGY

# Refuting Refugia?

Sandra Knapp and James Mallet

According to Charles Darwin, the origin of species was “the mystery of mysteries” (1). If so, then the Neotropical (Central and South American) forests, which harbor more plant species than the tropics of Asia and Africa combined (2), are the most mysterious of all. On page 122 of this issue, Wilf *et al.* (3) show that this massive diversification was active by the early Eocene, 52 million years ago. High plant species diversity in the Neotropics is clearly ancient.

Many biologists in the mid-20th century assumed that speciation occurred only in geographically separated populations. South America, with its continuous belt of rainforest and superfluity of species, might seem to contradict the idea of such “allopatric” speciation. In the 1960s, biologists applied the temperate-zone model of ice-age refugia to explain this enigma, rather than reevaluating the central role of allopatry in speciation theory (4).

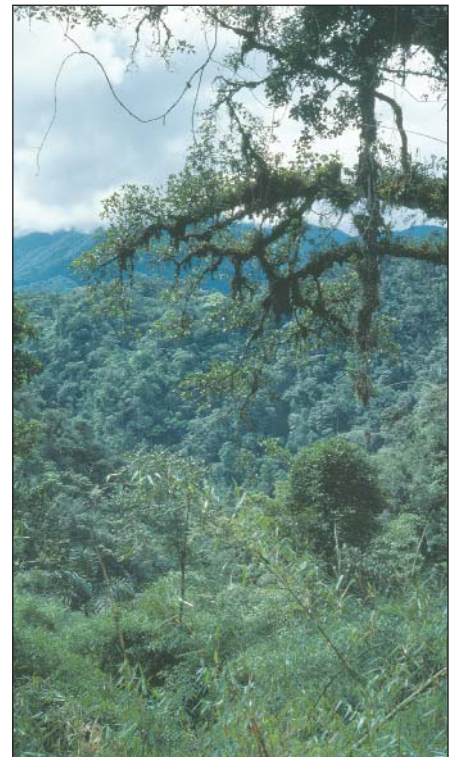
It had long been recognized that animal and plant ranges must have contracted while ice-sheets spread over the Northern Hemisphere. General cooling, sea-level reductions, and the locking up of much of the planet’s water in greatly extended polar ice caps are thought to have caused extensive aridity worldwide. Fossil sand dunes, pollen samples from sediment in high-elevation lake-beds, and “stone lines” (strata suggesting periods of high erosion expected during arid periods) all suggested that

arid climates may have prevailed in the Neotropics during much of the Pleistocene (1.64 million to 10,000 years ago).

Modern geographic patterns of differentiation and speciation across the Neotropical forest also hinted at past allopatry. If, during dry periods, rainforest became restricted to refuges scattered across tropical America, the allopatry deemed necessary for speciation could have occurred in waves throughout the Pleistocene. This

“refugium theory” was proposed to explain both geographic variation within species and overall high species diversity (4).

From the 1970s, the Neotropical refugium model accumulated many critics. If natural selection drives speciation, then gene flow will not affect diversification in areas that are much larger than the per-generation dispersal range of individuals; thus, “parapatric” speciation (geographic speciation while populations remain in contact, or “isolation by distance”) is as plausible as allopatric speciation (5). Furthermore, the geographic ranges of Amazonian birds may require no historical refugium explanation because random placement produces similar distributions (6). An analysis



**Where do all the species come from?** The diversity of the vast Neotropical rainforests has spawned numerous theories of origin, mostly based on climate change during the Pleistocene.

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## PERSPECTIVES

of hypothesized plant “refugia” showed that they corresponded closely with maxima of collecting activity (7), rather than representing a historical signal.

Today, the refugium hypothesis has been considerably modified (8) in the face of data on distributions, ages, and phylogenetic relationships of Neotropical taxa, and of recent paleoecological findings. There is little biological evidence for a set of forest refuges common to all organisms; in fact, the number and sizes of hypothesized refugia for any particular group appear to depend strongly on the dispersal abilities of the taxa examined (9).

Molecular evidence also suggests that divergence of many sister taxa preceded the Pleistocene (10), even though recent explosive speciation has been found in several Neotropical groups. Butterflies show evidence of species divergences at about 1 to 2 million years ago (11), and in some species-rich genera of flowering plants speciation occurred mostly in the last few million years (12). New alternative theories for speciation have been proposed. For example, Fjelds  (13) has argued that the Andes, after their formation during the Tertiary (65 million to 1.64 million years ago), became the species pump for the entire region, and that lowland forest acts merely as a “museum” for the diversity created in the highlands.

The geological evidence for periods of extreme aridity in the Neotropical rainforest is also now under attack (14). There is strong evidence for cyclical climatic change due to orbital forcing; what is less clear is whether “refugia,” islands of wet forest separated by dry savanna or semidesert, ever existed. According to Hooghiemstra, “the available evidence is far too inadequate to make any well-documented decision” about the existence of Pleistocene refugia (15).

Neotropical diversity clearly does depend on much more than the Amazon basin, although most debate about the exceptional plant species diversity of the Neotropics has centered on these seemingly continuous forests. The Andes, the most extensive mountain range in the world’s tropics, were certainly a factor promoting Neotropical species richness, and many plant species today are found only in the Andes. The late A.I. Gentry (16) pointed out two decades ago that families diverse in the Andes are not the same as those diverse in the Amazon; these patterns are rarely taken into account by those attempting to explain the excess of species-level diversity in the Neotropics.

Wilf *et al.* (3) show that high plant species diversity in the Neotropics was already present during the early Eocene. The Amazon at this time was almost certainly

as warm and as wet as it is today. The extremely high diversity of the Laguna del Hunco site in Patagonia, Argentina, apparently existed long before the main phase of Andean uplift (contradicting Fjelds ’s “museum” hypothesis), and long before the cooler and more turbulent climates of the Pleistocene.

A similarly rich flora at another Eocene Neotropical site (3) suggests large areas of forested habitat. There is fossil evidence for plant families that are now extinct (Casuarinaceae) or less diverse (Proteaceae) in the Neotropics, indicating that extinction and taxonomic turnover must be taken into account when trying to explain the species richness of the Neotropics. With high species diversity evident in the early Eocene, later climate change is neither sufficient nor necessary to explain Neotropical diversity.

Those who still believe that some form of allopatry was required for the current species diversity in the Neotropical rainforest may have to go back about 60 million years to find the requisite refugia. The debate over more recent Neotropical refugia may be irrelevant. A viable alternative to the refugium model is that a vast, relatively unbroken continental region will have sufficient genetic variation, ecological diversity, and isolation by distance to drive the evolution of the outstanding Neotropical diversity we see today.

Does all this matter? If we are to formulate strategies to nurture future evolutionary potential as well as conserve extant species, understanding the origins of

diversity is of the utmost urgency. Darwin was doubtless thinking of the diverse forests of South America when he wrote (1): “Although I do not doubt that [geographic] isolation is of considerable importance in the production of new species, on the whole I am inclined to believe that largeness of area is of more importance... not only will there be a better chance of favourable variations arising from the large number of individuals of the same species there supported, but the conditions of life are infinitely complex from the large number of already existing species” (p. 105). After three decades of debate about Pleistocene refugia, the findings of Wilf *et al.* (3) suggest that Darwin’s ideas may just turn out to be nearer the mark.

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## STRUCTURAL BIOLOGY

# A Glimpse into tmRNA-Mediated Ribosome Rescue

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**T**he basic mechanism of protein synthesis is conserved in all organisms. In reactions orchestrated by the ribosome and charged transfer RNAs (tRNAs), the genetic information in a messenger RNA (mRNA) is decoded into a protein sequence (1). However, occasionally the ribosomal machine stalls on an mRNA before polypeptide synthesis is complete. To rescue stalled ribosomes, eubacteria employ a molecule, called tmRNA, which functions both as a tRNA (2) and a mRNA

(3). It restarts protein synthesis and adds a peptide tag to the incomplete protein to target it for destruction by cellular proteases (see the figure) (4). On page 127 in this issue, Valle and colleagues report the cryo-electron microscopy (cryo-EM) structure of tmRNA in complex with the ribosome (5). The images of this rescue complex provide important new insights into tmRNA function.

During protein synthesis, a charged tRNA is delivered to the A site of the ribosome by EF-Tu, a guanosine triphosphatase (GTPase) that increases translational fidelity (1). EF-Tu senses proper matching of the tRNA anticodon and mRNA codon, trig-

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