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Increasing dominance of large lianas in Amazonian forests

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Ecological orthodoxy suggests that old-growth forests should be close to dynamic equilibrium, but this view has been challenged by recent findings that neotropical forests are accumulating carbon[1,2] and biomass[3,4], possibly in response to the increasing atmospheric concentrations of carbon dioxide[5,6]. However, it is unclear whether the recent increase in tree biomass has been accompanied by a shift in community composition. Such changes could reduce or enhance the carbon storage potential of old-growth forests in the long term. Here we show that non-fragmented Amazon forests are experiencing a concerted increase in the density, basal area and mean size of woody climbing plants (lianas). Over the last two decades of the twentieth century the dominance of large lianas relative to trees has increased by 1.7–4.6% a year. Lianas enhance tree mortality and suppress tree growth[7], so their rapid increase implies that the tropical terrestrial carbon sink may shut down sooner than current models suggest[8–10]. Predictions of future tropical carbon fluxes will need to account for the changing composition and dynamics of supposedly undisturbed forests.

Recent field studies[1,2,3] indicate that old-growth tropical forests are absorbing 1–2 Gt C yr^{-1}, but the mechanisms and stability of the tropical carbon sink, and its implications for the ecology of tropical vegetation, are highly uncertain. Shifts in functional composition and biodiversity are expected as a result of climate changes and increased CO_2 (refs 11, 12) but so far there is no evidence of widespread compositional change in old-growth forests. This absence of evidence might imply evidence of absence—or it could simply reflect our failure to monitor adequately forest behaviour, or even to examine existing data across sufficient spatial and temporal scales. Lianas in particular are ignored in forest inventories and models alike, in spite of their key functional roles. As structural parasites, lianas exert a much greater ecological effect than their size suggests, representing less than 5% of tropical forest biomass but up to 40% of leaf productivity[13]. They also suppress tree growth and encourage tree mortality, and affect the competitive balance among trees by disproportionately infesting some taxa and suppressing the regeneration and growth of non-pioneers[7]. Climbers respond strongly to increased CO_2 concentrations[14,15] and benefit from disturbance[7,16,17], and a biome-wide trend to increased tree turnover rates has been detected in old-growth forests[18] so increases in liana densities might be anticipated[19]. Here we assemble several unique,
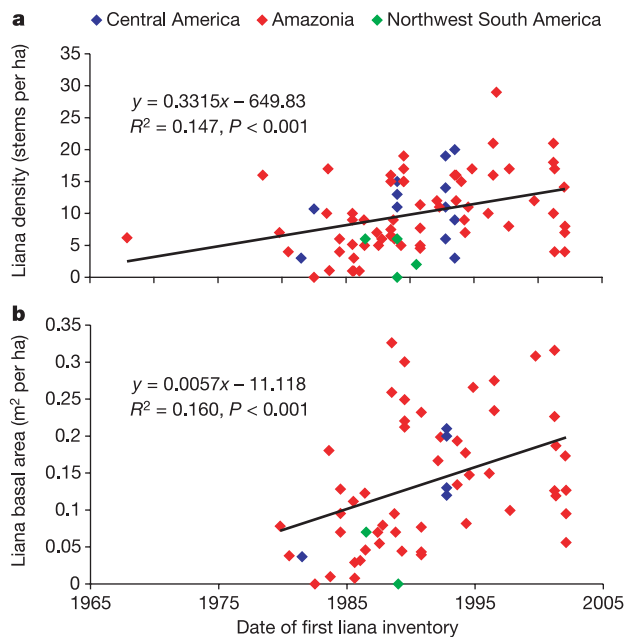
Figure 1 Structural importance of lianas over 10 cm in diameter in each neotropical site as a function of date of first inventory. **a**, Liana stem density in stems ha^{-1}; **b**, liana basal area in m^2 ha^{-1}. 'Central America' is Panama and tropical countries to the north; 'Northwest South America' is the Chocó bioregion, west of the Andes; 'Amazonia' is the Amazon river basin and contiguous forested zones of Guyana and eastern Brazil. Linear regressions are fitted to the Amazonian data.

Table 1 **Modelled changes in liana density and basal area**

Dependent variable	Source of variation				
	A. Environment only		B. Environment plus time		
	Adjusted R^2 (%)	F-value, d.f.	Δ Adjusted R^2 (%)	F-value, d.f.	T-value and significance for T in model
Liana stem density (ha^{-1})	35.3	3.83*, 26	+13.2	5.89***, 26	+2.93**
Liana basal area (m^2 ha^{-1})	27.2	2.94*, 26	+21.1	7.06***, 26	+4.42***
Number of lianas/number of trees	34.1	4.36*, 26	+12.4	6.66***, 26	+3.93***
Basal area of lianas/basal area of trees	34.6	3.60*, 26	+21.7	6.59***, 26	+4.80***
Mean liana basal area (cm^2)	0.3	1.04, 26	+5.5	1.54, 26	+1.55

*, $0.05 > P \geqslant 0.01$; **, $0.01 > P \geqslant 0.001$; ***, $P < 0.001$.
Multiple regression of large liana density and basal area (BA) at each initial census, modelled as a function of the environment, and the environment plus time. Environmental variables measured at North Peru, South Peru and Bolivian sites include climate (mean annual rainfall, seasonality), soil chemistry (pH, Ca, K, Mg, Na, P, Al), soil particle size distribution (sand, silt, clay), and hydrology (drainage and risk of water-logging). Soil variables were converted to principal components before multiple regression; time variable (T, in years) is the decimal date in which the liana parameter was first recorded in the plot. See Methods for details.

long-term, multi-regional data sets of liana and tree populations. We use them to test both the general hypothesis that the composition of old-growth tropical forests is changing over large scales, and the specific prediction that lianas are benefiting. We analyse 47 interior-forest sites in four Amazonian regions (North Peru, South Peru, Bolivia and Ecuador) where we are monitoring all woody plants of over 10 cm in diameter in 1-ha plots, and include published data from a further 37 neotropical sites. We find that the density and the basal area of large lianas have increased substantially over the last two decades of the twentieth century. The same trends are observed however liana populations are analysed.

First, we plot liana dominance as a function of each site's first inventory data (Fig. 1). There is broad scatter, reflecting forest variation and large sampling error at the plot scale[17], but a significant trend for late-censused neotropical sites to have greater liana dominance than early-censused sites. Analysis of covariance (ANCOVA) shows that this is not an artefact of spatial changes in sampling intensity—in models with lianas as the dependent variable, and region and year as independent variables, the year contributes significantly to both neotropical and Amazon liana stem density ($F_{1,69} = 15.30$, $P < 0.001$; $F_{1,53} = 14.37$, $P < 0.01$) and to neotropical and Amazon liana basal area ($F_{1,49} = 8.96$, $P < 0.01$; $F_{1,43} = 6.99$, $P < 0.02$). The increases in lianas as a function of first inventory dates are also unlikely to be artefacts of a change through time in the environmental characteristics of the forests sampled, because the date of first inventory contributes significantly to statistical models of liana density and basal area even after accounting for edaphic and climatic effects (Table 1).

Second, we analysed a different data set: the changes that

Table 2 **Linear trends in the structural importance of large lianas**

a Amazonian sites with a monitoring period of over 5 yr

Parameter	Annual rate of change in parameter (mean ± 95% CI)	Annual rate of change (proportion of site initial value)	Annual rate of change (proportion of site final value)
Liana stem density (ha^{-1})	+0.22 ± 0.11 ($n = 28$)	+4.03 ± 2.56%	+1.78 ± 0.82%
Liana basal area (m^2 ha^{-1})	+3.72 ± 1.16 × 10^{-3} ($n = 28$)	+4.58 ± 2.60%	+2.40 ± 0.62%
Liana stems as a fraction of tree stems	+3.45 ± 2.10 × 10^{-4} ($n = 28$)	+3.27 ± 2.10%	+1.70 ± 0.87%
Liana basal area as a fraction of tree basal area	+1.19 ± 0.53 × 10^{-4} ($n = 28$)	+4.05 ± 2.30%	+2.07 ± 0.71%
Mean basal area per liana stem (cm^2)	+1.03 ± 0.97 ($n = 27$)	+0.96 ± 0.66%	+0.65 ± 0.65%

b Amazonian sites with a monitoring period of over 5 yr analysed by region

Parameter	ANOVA, test of hypothesis of a regional cluster effect	Annual rate of change in parameter (mean ± 95% CI) for each region
Liana stem density (ha^{-1})	$F = 0.84$, $P = 0.48$	N. Peru +0.34 ± 0.25 ($n = 7$)
		S. Peru +0.19 ± 0.22 ($n = 12$)
		Bolivia +0.06 ± 0.41 ($n = 5$)
		Ecuador +0.28 ± 0.32 ($n = 4$)
Liana basal area (m^2 ha^{-1})	$F = 0.64$, $P = 0.60$	N. Peru +0.0049 ± 0.0022 ($n = 7$)
		S. Peru +0.0029 ± 0.0025 ($n = 12$)
		Bolivia +0.0042 ± 0.0029 ($n = 5$)
		Ecuador +0.0033 ± 0.0033 ($n = 4$)
Liana stems as a fraction of tree stems	$F = 0.95$, $P = 0.43$	N. Peru +6.34 ± 4.75 × 10^{-4} ($n = 7$)
		S. Peru +2.20 ± 3.91 × 10^{-4} ($n = 12$)
		Bolivia +2.10 ± 7.89 × 10^{-4} ($n = 5$)
		Ecuador +4.30 ± 5.70 × 10^{-4} ($n = 4$)
Liana basal area, as a fraction of tree basal area	$F = 0.62$, $P = 0.61$	N. Peru +1.79 ± 0.82 × 10^{-4} ($n = 7$)
		S. Peru +0.97 ± 1.21 × 10^{-4} ($n = 11$)
		Bolivia +0.80 ± 1.81 × 10^{-4} ($n = 5$)
		Ecuador +1.11 ± 1.46 × 10^{-4} ($n = 4$)
Mean basal area per liana stem (cm^2)	$F = 0.15$, $P = 0.93$	N. Peru +0.66 ± 2.92 ($n = 7$)
		S. Peru +0.96 ± 1.83 ($n = 11$)
		Bolivia +1.61 ± 1.90 ($n = 5$)
		Ecuador +1.20 ± 2.49 ($n = 4$)

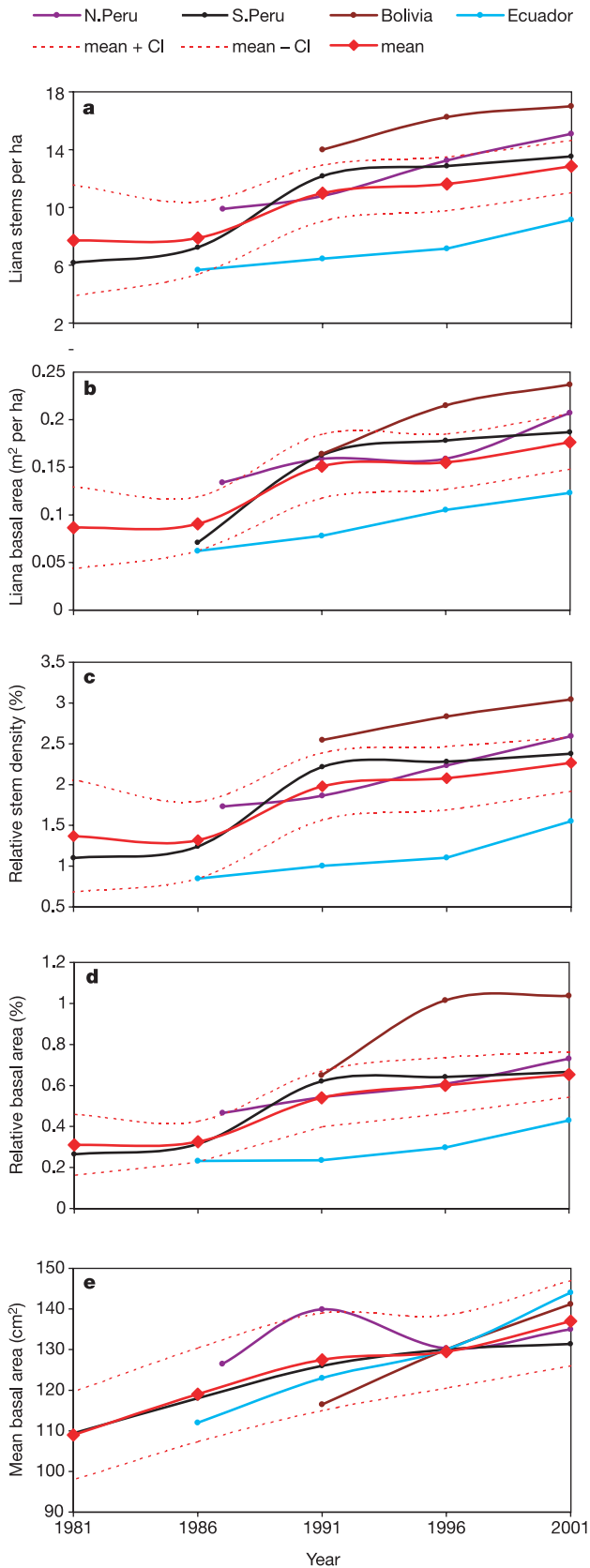ANOVA, analysis of variance. CI, confidence interval.

Figure 2 Changes through time of the importance of lianas over 10 cm in diameter in western Amazonia. **a**, Liana stem density in stems ha^{-1}; **b**, liana basal area in m^2 ha^{-1}; **c**, relative liana density as a percentage of tree stems; **d**, relative liana basal area as a percentage of tree basal area; **e**, mean basal area of each liana stem in cm^2. Graphs show 5-yr running means with 95% confidence intervals, with values plotted separately for North Peru, South Peru, Bolivia and Ecuador.

occurred through time within multi-census sites. Here we find consistently strong positive changes in measures of liana dominance (1.7–4.6% per year) and stem size (0.6–1.0% per year), evident across all regions (Table 2).

Finally, we assembled all available data from our four west Amazonian regions, that is single-census and multi-census data, to create running means across sites, and find similarly consistent patterns over the last two decades (Fig. 2). The year-on-year increase in mean values of large lianas is not driven by a few atypical sites or by the intrinsic liana richness of any one region, but is rather a general phenomenon across all five ecological parameters and all four regions (ANCOVA, with year as the continuous variable and region as fixed factor, shows that year contributes ($P < 0.01$) for all 20 combinations except for mean stem size in North Peru ($P > 0.05$)).

We examined the underlying dynamics of lianas in all plots censused three or more times, and find that the rates of both large liana growth and large liana loss have increased (comparing annual rate of basal area gain for lianas interval 1 versus interval 2, $t = -3.10$, $n = 21$, $P < 0.01$; annual rate of basal area loss for lianas interval 1 versus interval 2, $t = -2.66$, $n = 21$, $P < 0.02$), whereas growth rates have consistently exceeded loss rates (annual rate of basal area gain versus loss for lianas ≥ 10 cm in diameter for interval 1: $t = 4.19$, $n = 21$, $P < 0.001$; and for interval 2: $t = 2.38$, $n = 21$, $P < 0.05$). Thus the net increase in liana basal area was driven by high liana growth rates that have increased through time, and occurred in spite of an acceleration in the rate of liana mortality.

Tree basal area increased by 0.34 ± 0.20% a year in the 1980s and 1990s in Amazonia[3] but the increase in liana values has been much more rapid: the relative importance of large lianas has approximately doubled over a similar period across all sites (Fig. 2) and the annual rates of increase in liana density and BA within sites exceeded the rate of increase in tree BA by an order of magnitude (Table 2a).

Mature Amazonian forest plots have therefore undergone substantial change in functional composition. The increase in lianas has been concerted in the sense that it has occurred simultaneously over a wide spatial, climatic and edaphic range. We have shown that this is not an effect of changes in the kinds of forests sampled through time, and other potential artefactual explanations can also be ruled out (Supplementary Information). We can explore the generality of the changes indicated by the permanent plot data set with an independent data set from 70 old-growth lowland forests across the Neotropics. These are single-census 0.1-ha forest samples surveyed between 1971 and 1997, in which every tree and liana ≥ 2.5 cm in diameter was inventoried once (see Methods), thus sampling plants with basal area as low as about 6% of the smallest plants in our permanent plots. After controlling for climate and soil effects, the neotropical 0.1-ha data set confirms the temporal trends seen in the permanent plot data set: liana popu-
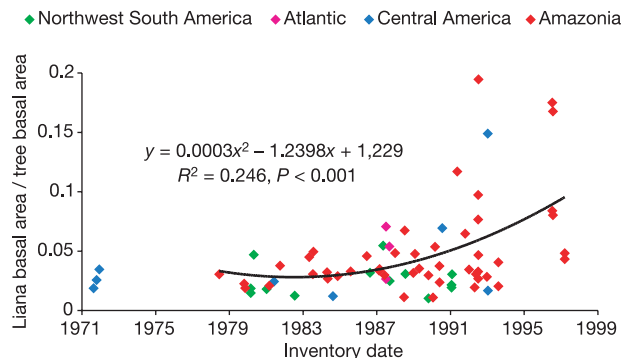

Figure 3 Relative dominance of lianas in neotropical 0.1-ha plots as a function of inventory date. Liana basal area is shown as a fraction of tree basal area for all stems over 2.5 cm in diameter. A polynomial curve is fitted for the Amazonian sites.

lations have become more dense, liana basal area has increased, the relative dominance of lianas has increased, and the size of individual lianas has increased (Fig. 3, and Supplementary Information Table 3). The degree of internal consistency within and between data sets across differing sample unit sizes, target variables, minimum plant sizes, climatic regimes, edaphic conditions, regional locations and spatial scales is a critical factor in assessing confidence in the changes. The results demonstrate a substantial increase in the density and relative dominance of lianas in western Amazonia, and available evidence suggests that this change in the structural and functional composition of forests has been even more widespread.

We asked what is driving this change? If regional climates have changed in western Amazonia or across the neotropics that could provide an explanation, but we failed to find convincing evidence for this (Supplementary Information). The documented increase in CO_2 concentrations is another possibility, because lianas respond strongly to CO_2 fertilization over the historical range of concentrations[15]. The direct impacts of CO_2 on photosynthesis may drive nonlinear compositional responses—for example, the relative rate of stimulation on liana growth may be particularly strong in deep shade[15], enhancing the likelihood of lianas reaching the sunlit canopy. Effects of increasing CO_2 on climber growth[14,15] could also be magnified in a positive feedback loop with the simultaneous increase in tree turnover[18] (more rapid turnover favours gap-specialist lianas which in turn accelerate tree mortality).

The increased liana load in trees may have a major impact on the Amazon carbon sink. The biomass of lianas themselves is usually small[7,20], but lianas can substantially suppress tree biomass[7,17]. We examined the relationship between liana infestation and subsequent tree mortality in the 13 sites where we have accurate records of liana–host relationships. Here, liana infestation of tree biomass is associated with a 39.6 ± 31.3% excess risk of tree mortality. The expected annual rate of increase in the amount of tree biomass mortality is estimated as 1.64 ± 1.38%, the product of the liana-associated excess tree mortality risk and the annual rate of increase in large lianas per unit of tree biomass in the sites. However climate models suggest that in east and south Amazonia moisture supply will become more seasonal[9,10], conditions which may favour lianas (Supplementary Information), so synergisms between climate change and increasing liana densities could magnify the impact of either process alone. Better understanding of these risks will require intensive field research to improve the liana-on-tree mortality functions and to begin including lianas within full tropical forest vegetation models and coupled carbon cycle/climate models.

The increase in liana density and biomass is the first evidence for a widespread functional shift in old-growth tropical forests. Regardless of the impacts on the carbon cycle, this has important implications for the biodiversity of tropical forests. First, if the increase is driven by increasing CO_2 concentrations it implies that the extensive tropical forests of the Cretaceous and Tertiary periods when CO_2 concentrations peaked at >2,000 p.p.m. (ref. 21) may have differed radically from today's in structure and function. Second, lianas and trees are differentiated phylogenetically[22] and by distinctive pollination and dispersal ecologies[23], so changed relative densities has knock-on consequences for conservation of plants and animals. Third, increased liana density has the potential to alter tree species composition because climber impacts on trees vary with host phylogeny and ecology[7,16]. Finally, the change in composition has direct societal and economic impacts, because lianas are valued less than trees by forest communities[24] and are major silvicultural pests for the tropical timber industry[7]. □

Methods

Study sites of 1 ha
We censused large liana populations in 47 sites spanning the climatic and edaphic gradients of western Amazonia[25] (Supplementary Information Appendix). 1-ha permanent plots were sited since as early as 1979 in old-growth forest and recensused every 2–5 yr, most recently in 2002 (eight sites) and 2001 (19 sites), yielding up to 19 yr of growth and dynamics data. Plot locations were constrained by the need for reasonable access (<10 km to nearest road or navigable river) and long-term protection, but are otherwise sited randomly or haphazardly within landform strata and are unbiased by sylvigenetic state[3,4]. The straight-line distance between all pairs of plot centroids ranges by four orders of magnitude (0.2–2,380 km), but intersite distance had no effect on liana change metrics (Supplementary Information). Distances to edges are 0.1 to ~3.6 km, where 'edge' is defined as a previously forested location where there has been an anthropogenic impact creating a canopy gap of ≥0.5 ha the effects of which are still apparent at the time of the first census. Edges were formed by farmers, research stations, tourist lodges and logging activities. We also searched the literature for published single-census large liana inventories in neotropical forests with ≥1,500 mm rain, including all except montane/cloud forests, small fragments (<1,000 ha), or with known human disturbance to forest structure.

Liana and tree measurements
Tree measurements and analysis follow RAINFOR protocols[25] (http://www.geog.leeds.ac.uk/projects/rainfor/). For lianas the diameter of each independently rooted climbing stem rooted within each plot and potentially over 10 cm wide was measured at a height of 1.3 m ($d_{1.3}$: all sites except 14, 15 and 25) and at the widest point within 2.5 m of the ground (d_{max}: all sites except 23, 24, 34 and 35), or at both points for most censuses (40 sites). We used the $d_{1.3}:d_{max}$ ratio of individual lianas to determine d_{max} of lianas at censuses where only $d_{1.3}$ was recorded. This procedure gives unbiased estimates, because the ratio of $d_{1.3}$ to d_{max} is independent of d_{max} (mean ± 95% confidence interval, CI, of r, the correlation between $d_{1.3}/d_{max}$ and d_{max} = 0.007 ± 0.080, $n = 28$). For both measurement methods we calculated the number of lianas and total basal area (the sum of cross-sectional stem areas) at each census. Lianas support greater biomass and productivity than trees of equal diameter[7,13,26], so stem density and basal area are not equivalent measures of functional importance between life-forms, but for each life-form basal area provides a close approximation to biomass[3,26,27]. For reasons of brevity we only report results based on $d_{1.3}$ measurements. Results based on d_{max} are presented in the Supplementary Information Tables 1 and 2 and are essentially equivalent.

Climate and soil data
Climate data were sourced directly from local records, or indirectly from interpolated maps[25,28], to derive mean annual rainfall and seasonality (consecutive months averaging <100 mm rain) for every neotropical site. At Bolivia and South and North Peru sites, soils were sampled from up to ten randomly chosen locations within each plot at 0–15 or 0–20 cm depth; samples were bulked, dried, and subsampled. Analysis followed standard ISRIC procedures[29]. Most sites have been visited at least five times, allowing assessment of hydrologic conditions on a scale of one (permanently water-logged) to ten (excessively draining).

Change in permanent plots
For within-site and within-region change analyses (Table 2) we included all interior-forest old-growth sites with at least two full censuses of trees and lianas over 10 cm in diameter more than 5 yr apart. For between-site change analyses (Fig. 1, Table 1) we included interior-forest old-growth plots with at least one full census of trees and lianas over 10 cm in diameter. To minimize effects of any asymmetric sampling of environments through time, we first excluded permanent swamp and white sand sites and then used principal components ordination analysis (PCA) to describe the major gradients in normalized and standardized soil variables in Peruvian and Bolivian sites, and then applied multiple regression to test the effects of the PCA factors, climate variables and the time variable on forest structure (Table 1), repeating the procedure with neotropical 0.1-ha sites (Supplementary Information Table 3). We report multiple-regression models with greatest adjusted-r^2 values and control for climate and edaphic effects by computing the variance explained by inventory date after accounting for the variance explained by environmental variables.

For the displays of change within- and between-sites (Fig. 2), we included interior-forest old-growth plots with at least one full census of trees and lianas. We used linear interpolation between each census to estimate structural values within sites and then derived cross-site 5-yr running means to smooth the effects of site-switching.

For comparisons of liana growth and loss within plots we split each monitoring period into intervals of similar length (first interval, 5.9 ± 0.7 yr; second interval, 6.0 ± 0.9 yr), so that turnover rates could be compared directly while controlling for possible effects of interval length on estimated mortality and growth rates.

Study sites of 0.1 ha
Inventories were completed by A. Gentry (58 sites) (http://www.mobot.org/MOBOT/Research/gentry/transects.html), R.V.M. and O.L.P. (6), and T.K., L.A. and M.S. (6). All scandent lianas and hemiepiphytes with d_{max} over 2.5 cm and non-climbing stems with $d_{1.3}$ over 2.5 cm were measured. See ref. 30 for detailed descriptions. All available neotropical old-growth forest samples with more than 1,500 mm rain were included, except montane/cloud forests, small fragments (less than 1,000 ha), or sites with known human disturbance to forest structure (extra-Amazonian sites also exclude island hurricane-impacted forests).

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Involvement of DARPP-32 phosphorylation in the stimulant action of caffeine

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Caffeine has been imbibed since ancient times in tea and coffee, and more recently in colas. Caffeine owes its psychostimulant action to a blockade of adenosine A_{2A} receptors[1], but little is known about its intracellular mechanism of action. Here we show that the stimulatory effect of caffeine on motor activity in mice was greatly reduced following genetic deletion of DARPP-32 (dopamine- and cyclic AMP-regulated phosphoprotein of relative molecular mass 32,000)[2]. Results virtually identical to those seen with caffeine were obtained with the selective A_{2A} antagonist SCH 58261. The depressant effect of the A_{2A} receptor agonist, CGS 21680, on motor activity was also greatly attenuated in DARPP-32 knockout mice. In support of a role for DARPP-32 in the action of caffeine, we found that, in striata of intact mice, caffeine increased the state of phosphorylation of DARPP-32 at Thr 75. Caffeine increased Thr 75 phosphorylation through inhibition of PP-2A-catalysed dephosphorylation, rather than through stimulation of cyclin-dependent kinase 5 (Cdk5)-catalysed phosphorylation, of this residue. Together, these studies demonstrate the involvement of DARPP-32 and its phosphorylation/dephosphorylation in the stimulant action of caffeine.

Striatal medium spiny neurons have an important role in the control of voluntary movements. A large subpopulation of these neurons project to the substantia nigra pars reticulata, the major
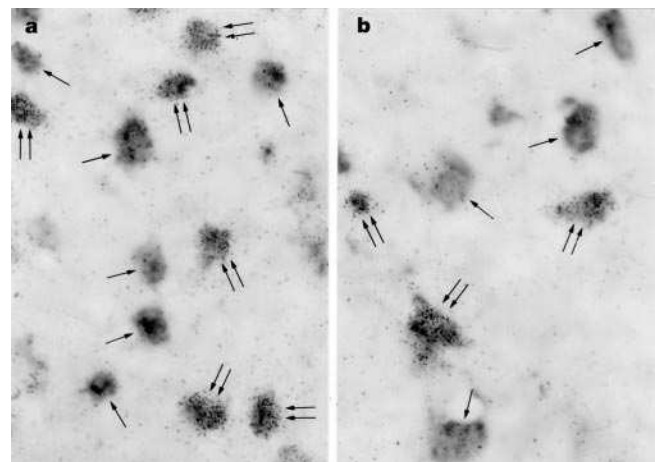

Figure 1 Emulsion autoradiogram illustrating co-expression of adenosine A_{2A} receptor mRNA (silver grains) and DARPP-32 mRNA (dark cells). Shown are subpopulations of medium spiny neurons in mouse (**a**) and rat (**b**) striatum. Single arrows indicate neurons that only express DARPP-32 mRNA. Double arrows indicate neurons that express both DARPP-32 mRNA and adenosine A_{2A} receptor mRNA.