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Human population in the biodiversity hotspots

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Biologists have identified 25 areas, called biodiversity hotspots, that are especially rich in endemic species and particularly threatened by human activities. The human population dynamics of these areas, however, are not well quantified. Here we report estimates of key demographic variables for each hotspot, and for three extensive tropical forest areas[1] that are less immediately threatened. We estimate that in 1995 more than 1.1 billion people, nearly 20% of world population, were living within the hotspots, an area covering about 12% of Earth's terrestrial surface. We estimate that the population growth rate in the hotspots (1995–2000) is 1.8% yr^{-1}, substantially higher than the population growth rate of the world as a whole (1.3% yr^{-1}) and above that of the developing countries (1.6% yr^{-1}). These results suggest that substantial human-induced environmental changes are likely to continue in the hotspots and that demographic change remains an important factor in global biodiversity conservation. The results also underline the potential conservation significance of the continuing worldwide declines in human fertility and of policies and programs that influence human migration.

In 1988, ecologist Norman Myers introduced the term 'biodiversity hotspots' to distinguish a global set of high-priority terrestrial ecoregions for conservation[2]. Myers and others argue that, because their 25 hotspots are high in species endemism and low in pristine vegetation (< 30% remaining), wise conservation investments in these ecoregions could help minimize future extinctions[2–4]. Primatologist Russell Mittermeier subsequently developed a complementary concept, the 'major tropical wilderness areas'[5]. These three areas of tropical forest (Upper Amazonia/Guyana Shield, the Congo Basin, and the New Guinea/Melanesian Islands) are the most pristine of all terrestrial ecoregions exhibiting a high degree of species endemism. Together they cover 6.3% of Earth's terrestrial surface, an area larger than the United States or China. Myers, Mittermeier and others propose a strategy of conservation investments in these areas as a back-up strategy for the hotspot approach[1]. By using mapped world distributions of humans (Fig. 1), various census sources and ecoregional boundary data, we calculated population density and growth rates for each of the biodiversity hotspots and major tropical wilderness areas (see Methods).

We estimate that in 1995 population density in the hotspots was 73 people km^{-2}, a figure 71% greater than that of the world as a whole (excluding ice- or rock-covered land). We found that 16 of the 25 hotspots (Fig. 2a) have population densities at or above the world average (42 people km^{-2}). According to our estimates, from 1995 to 2000, human population was still growing in all but one of the hotspots (the Caucasus), with 19 of the hotspot populations

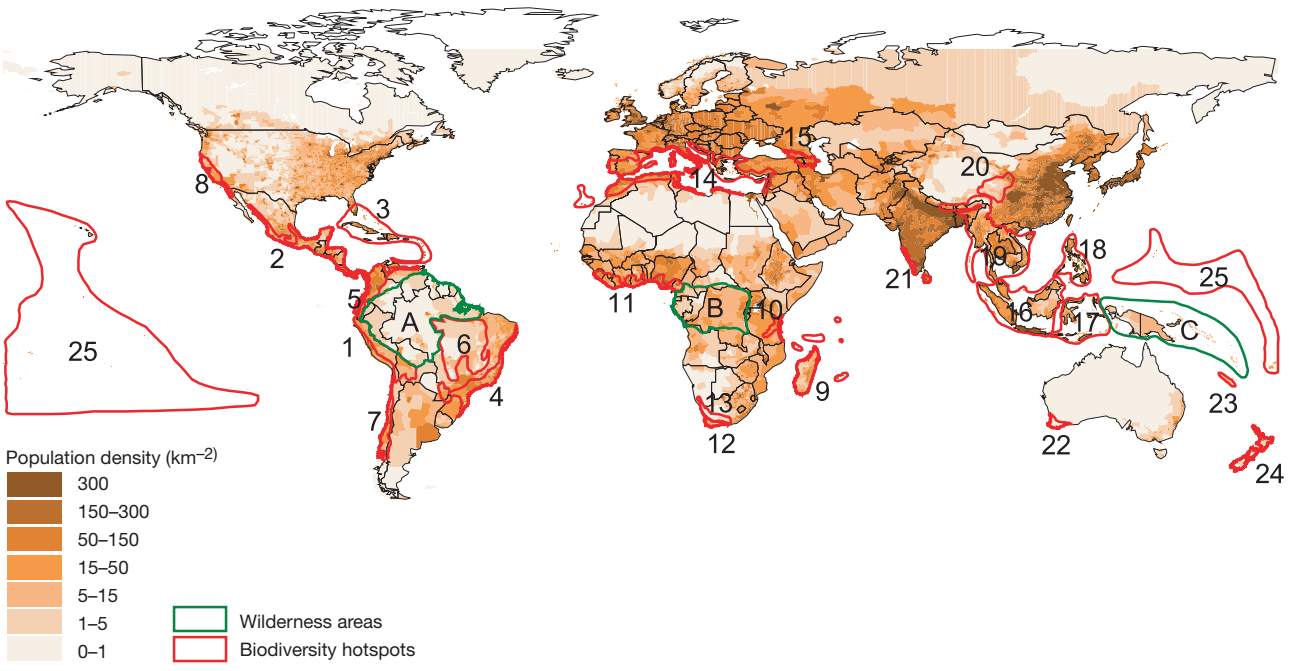

Figure 1 World population density (1995) and the 25 biodiversity hotspots (outlined in red, numbered), and three major tropical wilderness areas (outlined in green, lettered). Hotspots: (1) Tropical Andes; (2) Mesoamerica; (3) Caribbean; (4) Atlantic Forest Region; (5) Chocó-Darién-Western Ecuador; (6) Brazilian Cerrado; (7) Central Chile; (8) California Floristic Province; (9) Madagascar; (10) Eastern Arc Mountains and Coastal Forests of Tanzania and Kenya; (11) West African Forests; (12) Cape Floristic Region; (13) Succulent Karoo; (14) Mediterranean Basin; (15) Caucasus; (16) Sundaland; (17) Wallacea; (18) Philippines; (19) Indo-Burma; (20) Mountains of South-Central China; (21) Western Ghats and Sri Lanka; (22) Southwest Australia; (23) New Caledonia; (24) New Zealand; and (25) Polynesia and Micronesia. Major tropical wilderness areas: (A) Upper Amazonia and Guyana Shield; (B) Congo River Basin; and (C) New Guinea and Melanesian Islands.

growing more rapidly than that of the world as a whole (Fig. 2b). Although population growth rates were, in general, highest in the 19 hotspots wholly within developing countries, growth rates in the hotspots within developed countries were in most cases substantially higher than the worldwide average for developed regions (0.3% yr^{-1}).

In 1995, nearly 75 million people (1.3% of world population) were living within the three major tropical wilderness areas, representing an average density of about 8 people km^{-2}. (Area boundaries enclose several major cities.) These areas are experiencing population growth at a rate of 3.1% yr^{-1}, which is more than twice the global rate.

If population numbers are examined in isolation of other factors, the three hotspots with the most elevated risks, as assessed by high human population density, are the Western Ghats/Sri Lanka, Philippines and Caribbean hotspots. Chocó-Darién-Western Ecuador, Tropical Andes and Madagascar head a list of hotspots facing elevated risks on the basis of rapid population growth alone. Notably, the latest hotspot analysis by Mittermeier *et al*. concludes that the Philippines, Caribbean and Madagascar hotspots appear to be the highest-priority of these ecoregions on the basis of their extreme endemism and the intense packing of species into a much reduced area of original vegetation[6].

Human population variables are imperfect indicators of risk to biodiversity. Population density figures, for example, obscure patterns of population distribution within areas. Roughly 90% of the population of the southwest Australia hotspot lives in and around Perth, a single metropolitan area covering less than 2% of the ecoregion; however, such uneven distribution does not negate risk to biodiversity. There is considerable evidence of the capacity of urban populations to alter ecosystems, which are sometimes more than 100 km away, through demand for wood fuel (principally in developing countries), water, food (including wild foods), waste disposal and recreation (mostly in developed countries)[7,8].

Another problem is that disturbance caused by humans can occur in the absence of widespread human settlement. This is the frequent result of over-logging, burning, grazing, mining and commercial hunting that have extracted or degraded natural resources, abetted biological invasion or polluted soil and water resources[9]. Population density remains low, for example, in the most arid hotspot, the Succulent Karoo, which experiences heavy grazing and the over-harvesting of its flora for the international trade in ornamental plants.

Population growth rates can also be misleading indicators of risk to biodiversity. Because growth rates are calculated as the annual percentage change in a population, low rates of growth in dense populations add more individuals than much higher rates of growth in sparse populations. Population growth rates mask spatial distributions of growth and the trend of that rate. And both density and growth rates hide the culture, affluence and technology of the numbers of people they represent.

Despite these caveats, however, population trends in the biodiversity hotspots and major tropical wilderness areas indicate a high risk that habitats will continue to degrade as ecosystems dominated by humans expand and species become extinct in the world's most biologically diverse terrestrial regions. Results of the analysis also suggest that, whatever species conservation strategies ultimately emerge, conservation scientists and policymakers should take human population dynamics into account. Especially relevant are trends and potential changes in population growth, density and migration, and the social and economic factors known to influence population variables. One hopeful sign for the conservation of biodiversity is that declines in human fertility are gradually slowing population growth worldwide. □

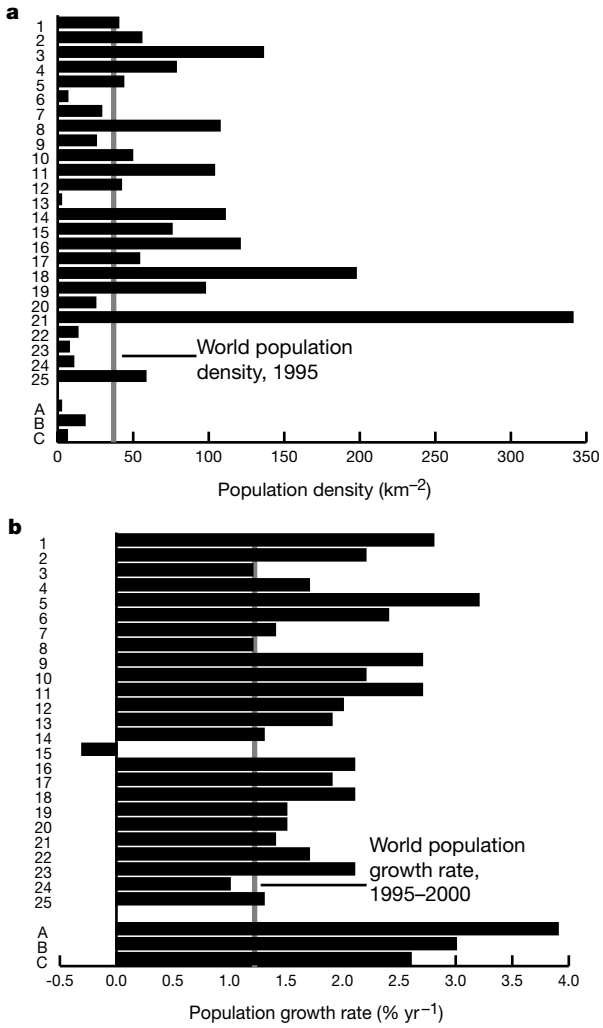

Figure 2 Human population densities (**a**) and annual growth rates (**b**) in the 25 global biodiversity hotspots (1–25; see map for names and locations, Fig. 1) and the three major tropical wilderness areas (A, B, C).

Methods

Population density

We estimated population densities for biodiversity hotspots and major tropical wilderness areas (ecoregions) using the *Gridded Population of the World, 1995*, a geographic information systems (GIS) layer developed by geographers at the National Center for Geographic Information and Analysis, University of California, Santa Barbara[10]. The authors of this layer call attention to numerous sources of potential error in these data, including extrapolation from census-year estimates to 1995 projections, the mapping of census geographical boundaries and census estimates themselves. For census data in most industrialized countries, demographers regularly assume an error (most often an undercount) of less than 3% of the actual population[11]. Errors exceeding 10% occasionally occur in censuses in the poorest countries, particularly those experiencing political instability. Moreover the populations enumerated vary from country to country, with some countries including, for example, military personnel living outside the country.

Population growth

We partitioned hotspots into countries and sub-national political divisions (provinces or states), used available growth rates or census and projection data to determine the growth of each unit, and then calculated average growth rates for the composite ecoregion. Data on a provincial level were used in ecoregions covering parts of Argentina, Australia, Brazil, Bolivia, Colombia, China, Ecuador, France, India, Indonesia, Mexico, Panama, Peru, South Africa, Spain, Turkey, United States and Venezuela. Where provincial data were unavailable or unnecessary (where the entire country fell within the hotspot), country population growth rates were obtained from estimates generated by the United Nations Population Division[12]. These United Nations data are also the source for 1995 world population density and 1995–2000 world population growth rates.

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1. Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B. & Kent, J. Biodiversity hotspots for conservation priorities. *Nature* **403,** 853–858 (2000).
2. Myers, N. Threatened biotas: hotspots in tropical forests. *Environmentalist* **8,** 178–208 (1988).

3. Myers, N. The biodiversity challenge: expanded hot-spot analysis. *Environmentalist* **10,** 243–256 (1990).
4. Mittermeier, R. A., Myers, N., Thomsen, J. B., da Fonesca, G. A. B. & Olivieri, S. Biodiversity hotspots and major tropical wilderness areas: approaches to setting conservation priorities. *Conserv. Biol.* **12,** 516–520 (1998).
5. Mittermeier, R. A. in *Biodiversity* (eds Wilson, E. O. & Peter, F. M.) 145–154 (National Academy Press, Washington, DC, 1988).
6. Mittermeier, R. A., Myers, N., Robles Gil, P. & Mittermeier, C. G. *Hotspots: Earth's Biologically Richest and Most Threatened Ecosystems* (Cemex, Mexico, D.F., 1999).
7. Repetto, R. *The "Second India" Revisited: Population, Poverty, and Environmental Stress Over Two Decades* (World Resources Institute, Washington, DC, 1994).
8. Myers, N. in *Population—The Complex Reality* (ed. Graham-Smith, F.) 117–135 (Royal Society, London, 1994).
9. Stedman-Edwards, P. *The Root Causes of Biodiversity Loss: An Analytical Approach* (Worldwide Fund for Nature, Washington, DC, 1997).
10. Tobler, W., Deichmann, U., Gottsegen, J. & Maloy, K. *The Global Demography Project* Tech. Rep. No. 95–6 (National Center for Geographic Information Analysis, Univ. California, Santa Barbara, 1995).
11. Newman, J. L. & Matzke, G. E. *Population: Patterns, Dynamics, and Prospects* (Prentice-Hall, Englewood Cliffs, 1984).
12. U.N. Population Division *World Population Prospects: the 1998 Revision* (United Nations, New York, 1998).

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Identification of sleep-promoting neurons *in vitro*

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The neurons responsible for the onset of sleep are thought to be located in the preoptic area[1–3] and more specifically, in the ventrolateral preoptic nucleus (VLPO)[4–6]. Here we identify sleep-promoting neurons *in vitro* and show that they represent an homogeneous population of cells that must be inhibited by systems of arousal during the waking state. We find that two-thirds of the VLPO neurons are multipolar triangular cells that show a low-threshold spike. This proportion matches that of cells active during sleep in the same region[6]. We then show, using single-cell reverse transcriptase followed by polymerase chain reaction, that these neurons probably contain γ-aminobutyric acid (GABA). We also show that these neurons are inhibited by noradrenaline and acetylcholine, both of which are transmitters of wakefulness[3,7,8]. As most of these cells are also inhibited by serotonin but unaffected by histamine, their overall inhibition by transmitters of wakefulness is in agreement with their relative inactivity during waking with respect to sleep[6]. We propose that the reciprocal inhibitory interaction of such VLPO neurons with the noradrenergic, serotoninergic and cholinergic waking systems to which they project[5,9,10] is a key factor for promoting sleep.

Intracellular recordings in slices revealed only two cell types within the VLPO. Of 102 recorded cells, most (n = 70, 68.6%) were characterized by a potent low-threshold spike (LTS)[11] (asterisk and inset in Fig. 1a, LTS cells) that was calcium dependent, as it persisted in tetrodotoxin (TTX, 1 μM) and was eliminated (n = 3) by nickel (200–500 μM). However, we found no evidence for an intrinsic rhythmicity driven by the LTS[11] in these cells. The second, less numerous cell type (n = 32, 31.4%) lacked an LTS (Fig. 1b, non-LTS cells) and was usually characterized by a more or less prominent rectification apparent upon depolarization from a hyperpolarized level (Fig. 1b, arrow). Basic membrane parameters, such as resting potential, membrane input resistance and action potential width did not differ between the two cell types. Injection of the intracellular tracer neurobiotin into VLPO neurons indicated that whereas both cell types were medium-sized (LTS cells, n = 14; mean large diameter ± s.d., 19.1 ± 2.0 μm; mean small diameter, 13.4 ± 1.3 μm; Fig. 1c, d; non-LTS cells, n = 6; 21.3 ± 3.1 μm versus 11.8 ± 1.3 μm, respectively; Fig. 1e, f), their shapes and dendritic arbours were completely different. All LTS cells were triangular (Fig. 1d) and multipolar (mean number of primary dendrites: 3.0 ± 0.0, n = 14), whereas non-LTS cells were fusiform (Fig. 1f) and bipolar (1.8 ± 0.4, n = 6).

The high percentage (68%) of LTS cells in the VLPO matches that of cells active during sleep in this region[4,6] and indicates that the LTS cells may correspond to these sleep-active cells. To test this proposal we measured the effects of noradrenaline, an important transmitter of wakefulness[3,7,8], and found that 18 out of 20 LTS cells (Fig. 2a, c) were hyperpolarized by noradrenaline (two were depolarized), whereas all (n = 8) non-LTS cells were depolarized (Fig. 2b, c). These results indicate that the LTS cells in the VLPO should be inhibited during waking, when noradrenaline is preferentially released[3,7,8], and thus are well suited to correspond to the sleep-active cells recorded *in vivo*[1,12,13]. Non-LTS cells, in contrast, are not well qualified for that role and will not be considered further here.

The results described above were obtained from intracellular recordings using sharp electrodes. We wanted to test whether VLPO cells are inhibited by noradrenaline in a condition closer to the *in vivo* situation, that is, with minimal perturbation of the cells' properties. We therefore used infrared videomicroscopy[14] to record extracellularly from VLPO triangular multipolar neurons (Fig. 3a) in a loose-attached cell configuration[15]. All neurons (n = 9) tested in this way were inhibited by noradrenaline (Fig. 3b, c). We then tested whether neurons inhibited by noradrenaline were also inhibited by acetylcholine, another important transmitter of arousal[3,7,8]; in every case (n = 5), these neurons were inhibited by both transmitters (Fig. 3d, e). In addition, the effects of both transmitters were postsynaptic, as they persisted (n = 2) in a high magnesium (10 mM)/low calcium (0.1 mM) solution.

We also investigated the two other transmitters (serotonin and histamine) usually associated with arousal[3,8]. Serotonin (100 μM, n = 10), like acetylcholine, inhibited the majority of cells (7 out of 10) previously inhibited by noradrenaline (Fig. 3f, g) and excited only a minority (3 out of 10). Both effects persisted (respectively, n = 2 and n = 1) in a high magnesium/low calcium solution. In contrast, histamine (100 μM, n = 5), which was also tested on neurons inhibited by noradrenaline, had no inhibitory or excitatory effect (not shown).

To establish the possible functional role of the LTS cells we needed to identify their neurotransmitter. Most of the VLPO cells, retrogradely labelled from the histaminergic tuberomammillary nucleus[5], the noradrenergic locus coeruleus[9] or the cholinergic magnocellular preoptic nucleus[10], are immunoreactive to glutamic acid decarboxylase (GAD) and thus contain GABA. We investigated the expression of GAD in LTS cells using single-cell reverse transcriptase followed by polymerase chain reaction (RT–PCR)[16–19]. In addition to GAD65 and GAD67, the synthesizing enzymes for GABA, we examined the expression of choline acetyltransferase