LETTERS

Low beta diversity of herbivorous insects in tropical forests

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Recent advances in understanding insect communities in tropical forests[1,2] have contributed little to our knowledge of large-scale patterns of insect diversity, because incomplete taxonomic knowledge of many tropical species hinders the mapping of their distribution records[3]. This impedes an understanding of global biodiversity patterns and explains why tropical insects are underrepresented in conservation biology. Our study of approximately 500 species from three herbivorous guilds feeding on foliage (caterpillars, Lepidoptera), wood (ambrosia beetles, Coleoptera) and fruit (fruitflies, Diptera) found a low rate of change in species composition (beta diversity) across 75,000 square kilometres of contiguous lowland rainforest in Papua New Guinea, as most species were widely distributed. For caterpillars feeding on large plant genera, most species fed on multiple host species, so that even locally restricted plant species did not support endemic herbivores. Large plant genera represented a continuously distributed resource easily colonized by moths and butterflies over hundreds of kilometres. Low beta diversity was also documented in groups with differing host specificity (fruitflies and ambrosia beetles), suggesting that dispersal limitation does not have a substantial role in shaping the distribution of insect species in New Guinea lowland rainforests. Similar patterns of low beta diversity can be expected in other tropical lowland rainforests, as they are typically situated in the extensive low basins of major tropical rivers similar to the Sepik–Ramu region of New Guinea studied here.

Locally coexisting species (alpha diversity) represent a large proportion of the regional species pool (gamma diversity) for many of the few tropical insect taxa for which distributions are known[4–6]. For instance, a single lowland rainforest site hosted 37% of all butterfly species of Borneo[6]; another hosted 40% of all taxonomically described fruitfly species of Papua New Guinea[4]. This pattern, implying a low rate of spatial change in species composition (beta diversity), is at variance with the high beta diversity of samples obtained from tropical forest canopies where a majority of species occur at single sites[7]. Overestimates of beta diversity can result from inadequate sampling of numerous rare species. On the other hand, relying on the taxonomically known species might underestimate the extent of beta diversity, as widespread species tend to be described first[8]. Quantitative studies of insect communities replicated on a regional scale are needed to resolve the debate.

Beta diversity of tropical herbivores has been examined in relation to latitudinal, altitudinal, disturbance and climatic gradients[3,9,10]. Not surprisingly, these studies confirmed high species turnover among sites as they comprised very different vegetation types. Even so, the relative influence of plant species composition, herbivore host specificity, or herbivore dispersal on the large-scale distribution of tropical insects remains unknown. Such knowledge is needed to understand the roles of historical and contemporary ecological processes in maintaining tropical diversity[11], to predict species extinction after habitat fragmentation, and to design systems of protected natural areas[12].

We sampled ~75,000 caterpillars (Lepidoptera) from 370 species feeding on plant species from four diverse genera (*Ficus*, *Psychotria*, *Syzygium* and *Macaranga*) across eight sites situated within a 500×150 km matrix of contiguous, largely undisturbed lowland rainforest in Papua New Guinea (Fig. 1). Study sites were evenly distributed across the Ramu and Sepik river basins and are characterized by relatively uniform altitude, climate, soil and vegetation. The comparison of caterpillar communities across a matrix of host plant species and sites represents the first attempt to assess insect beta diversity while controlling for the effects of host plant availability, altitude, rainfall, and habitat type and fragmentation. This survey was partially replicated using ambrosia beetles (Coleoptera: Scolytinae and Platypodinae) collected from four tree species at three sites, and fruitflies (Diptera: Tephritidae) attracted to lure traps from diverse rainforest vegetation at four sites.

The four plant genera were represented by 175 species across the study area (Supplementary Appendix 1). Similarity in species composition between the study sites was very high for *Ficus* and low for *Psychotria* and *Syzygium*, but in neither case did similarity decline significantly with distance between the sites. Decay of similarity in species composition was significant only for *Macaranga* (Fig. 2a and Supplementary Table 1). Similarity of caterpillar communities feeding on a particular plant species declined significantly with distance between sites in nine of the eighteen plant species sampled for herbivores. Similarity decreased gradually with geographical distance so that the proportion of species shared between sites remained >50% for distances up to 500 km (Fig. 2b and Supplementary Table 1). Likewise, samples of ambrosia beetles reared from particular plant species shared >60% of species (Fig. 2c) and fruitfly communities remained virtually constant (Fig. 2d) for distances up to 950 km.

The insect species collected at each study site were compared with the species pool known to occur in a 20×30 km area also including the town of Madang (Fig. 1). This is a relatively well-known regional fauna at the eastern boundary of our study area, intensely studied for >10 yr[4,13,14]. The proportion of caterpillar species recorded from

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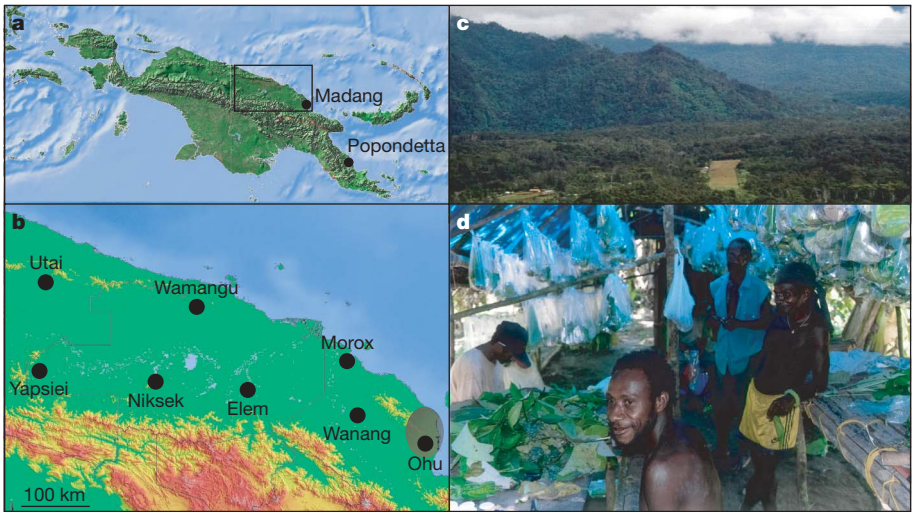

Figure 1 | Study sites and field techniques of insect rearing. **a**, The study is located in the basins of the Sepik and Ramu rivers within a 75,000 km^2 area of lowland terrain with continuous rainforest and wetland vegetation, and at an additional site (Popondetta) in the Papuan Peninsula. **b**, The location of eight lowland (<500 m above sea level) rainforest study sites with pair-wise distances ranging from 59 to 513 km. Madang area, including also the Ohu site, is marked by an ellipse. **c**, A typical study site (Yapsiei) including a village with an airstrip surrounded by large tracts of rainforest. **d**, A field laboratory rearing caterpillars at one of the study sites.

each site also known from Madang decreased linearly with distance from Madang. However, the slope of the relationship was low so that even the samples 500 km from Madang included >60% of Madang species. All samples of ambrosia beetles and fruitflies comprised >75% of species known also from the Madang area, irrespective of their distance from that area (Fig. 3).

The probability $C(d)$ of two caterpillars drawn at random from the same host species d kilometres apart belonging to the same species (Supplementary Fig. 1) is strongly influenced by common species and, as such, measures the turnover of dominant species between communities, a different aspect of beta diversity than the proportion of shared species. There was no decline in $C(d)$ with distance for $d = 59$–513 km. The values reported here (>0.1) are remarkably high, particularly when compared with neotropical rainforest tree communities where $C(d) < 0.01$ (ref. 15).

Hubbell's neutral model[16], where only dispersal and speciation affect species distribution, predicts that $C(d)$ declines linearly with log-distance over a wide range of distances. This relationship was shown for rainforest tree communities[15]. The lack of a distance effect on $C(d)$ suggests that dispersal limitation may not be important in structuring caterpillar communities at this spatial resolution. The weak effect of distance on community similarity is consistent with the broad distribution of species across the study area. Most of the species sufficiently abundant for analysis were collected at the majority of study sites not only for caterpillars but also for ambrosia beetles and fruitflies (Fig. 4 and Supplementary Fig. 2).

The Sepik, a major tropical river representing the only large discontinuity in the rainforest ecosystem of the study area, is probably not a barrier to lepidopteran dispersal as there was no difference in similarity between caterpillar samples taken on either side of the river

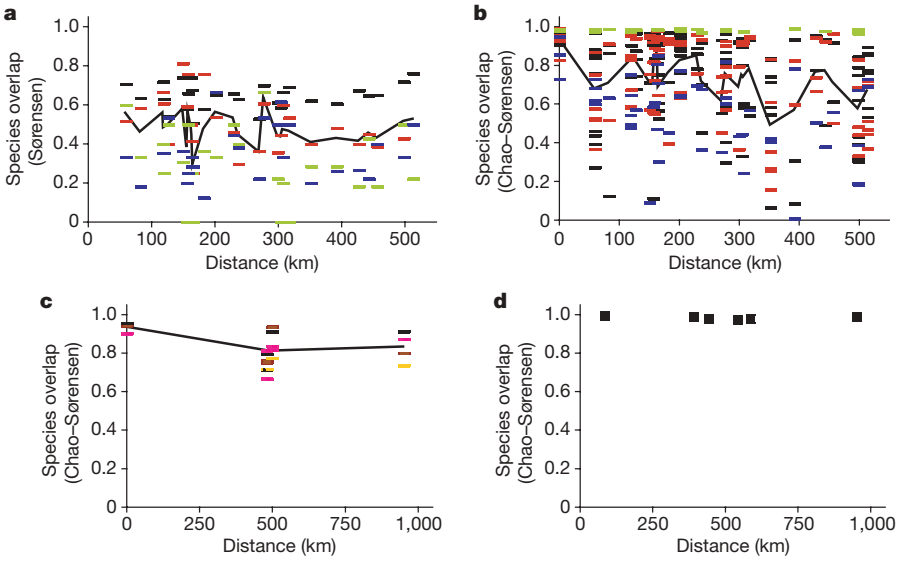

Figure 2 | Similarity of plant, caterpillar, ambrosia beetle and fruitfly assemblages as a function of geographical distance. **a**, Sørensen similarity of species composition in each of four plant genera among all possible pairs of study sites is shown. **b**, **c**, Chao–Sørensen similarity for caterpillar (**b**) and ambrosia beetle (**c**) assemblages feeding on particular plant species is shown for all plant species at all possible pairs of sites where they were sampled. **d**, Chao–Sørensen similarity for fruitflies from diverse forest vegetation is shown for all possible pairs of study sites. Markers denote host species of *Ficus* (black), *Macaranga* (red), *Psychotria* (green), *Syzygium* (blue), *Artocarpus* (brown), *Leea* (yellow), *Nauclea* (violet) and mixed forest vegetation (solid squares). Lines connect average values in different distance categories. Methods and Supplementary Table 2 list the plant species.

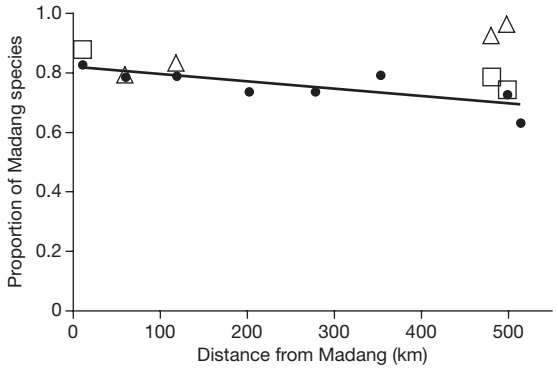

Figure 3 | Overlap in species composition between the Madang regional species pool and insect assemblages at each of the study sites as a function of their distance from Madang. The proportion of species known to occur also in the Madang area is reported for the caterpillar communities feeding on four plant genera (filled circles), ambrosia beetles feeding on four plant species (open squares) and fruitfly species sampled from diverse forest vegetation (open triangles) at each of the study sites. The regression line was fitted to data on caterpillars only (Pearson $r = 0.78$, $P < 0.01$, $n = 8$ sites).

(Chao–Sørensen index 0.79 ± 0.05) when compared to equidistant sites on the same side of the river (0.78 ± 0.04, paired t-test, $P > 0.8$, $N = 19$). The importance of large rivers as barriers to dispersal in lowland rainforests continues to be debated, as the evidence is equivocal at least in the Amazon Basin[17,18].

Contemporary estimates of beta diversity can be placed in the context of historical changes in climate, topography and vegetation. Our study area is characterized by complex geological history, sea incursions and vegetation change (see Methods). The broad geographical distribution of insect herbivores suggests that herbivorous insects effectively track their plant resources even across such a very dynamic landscape.

Host specificity was not correlated with geographical distribution in Lepidoptera (Fig. 4). This is probably because most Lepidoptera were clade specialists feeding on multiple congeneric plant species as opposed to feeding on only a single host species. The maximum possible geographical span for a particular herbivore species is a function of the combined distributions of all recorded hosts. It amounted to the entire 500-km span for all common lepidopteran species analysed, so that the potential distribution of Lepidoptera was not limited by the distribution of particular host plant species (Supplementary Results).

Plant species with limited geographical distribution did not support many specialized herbivores. Although four out of five *Psychotria* species sampled for caterpillars had restricted distributions (Supplementary Table 2), all were dominated by one or more of only three crambid species together representing 66–99% of all caterpillars feeding on *Psychotria* at each site. This is illustrative of a general pattern, as there was no correlation between the geographical distribution of particular host species and the average geographical distribution of their lepidopteran herbivores (Supplementary Fig. 3).

The number of lepidopteran species (S) increases as a power function of the number of hosts and the number of sites: $\log S = a + b\log(\text{host species}) + d\log(\text{sites})$ (Supplementary Fig. 4). This simple model combines a power function describing species–area relationships[19] and another describing the accumulation of herbivore species with increasing host plant diversity[14], enabling the prediction of herbivore species richness on diverse vegetation across geographical areas.

This report of low insect beta diversity across a large area of tropical forest points to the need for comparative data from other major forest ecosystems. Species range sizes are known to decrease with latitude in various taxa, implying higher beta diversity in the tropics (Rapoport's rule), but this trend has yet to be documented in insects[20].

Large plant genera represent a continuous resource for caterpillars that is readily colonized across large areas of lowland rainforests. Ambrosia beetles and fruitflies also exhibited low beta diversity, such that dispersal limitation seems to be unimportant for at least three herbivore guilds up to distances of 500–1,000 km. Insect species were broadly distributed across habitat discontinuities such as large rivers, and across historically disjunct geological terranes. A complete description of beta diversity need be supplemented by data on specialized herbivores from species-poor plant genera with limited geographical distribution, as these might produce higher estimates of beta diversity. For instance, most of the >700 monotypic genera in the flora of New Guinea[21] have poorly known geographical distributions and herbivore communities. Furthermore, we only studied relatively common species of trees. This bias may not be serious, because at least host specificity, one of the potential determinants of beta diversity, showed no relationship with the local abundance of host plants[22]. Rare insect species and their contribution to beta diversity are also a concern, but prove difficult to study.

Our beta diversity estimates obtained from samples of communities in different locations are in general agreement with low beta diversity estimates based on regional species pools[4–6]. This lends further support to relatively low global estimates of insect diversity at <10 million species[23–25]. Previous estimates of global insect species richness derived from the number of plant species and local species richness of herbivore communities were approximately doubled to account for beta diversity[23] or failed to consider geographical turnover among herbivore communities on conspecific trees[24]. Expanding the scope of sampling from a single location to distances up to 500 km between sites approximately doubled the number of unique lepidopteran species per plant species (Supplementary Figs 4 and 5).

Steep environmental gradients can show high turnover in herbivore communities even on the same plant species. For instance, *Ficus* trees at our lowland sites shared very few lepidopteran species with conspecific trees at 1,800 m above sea level in the New Guinea central

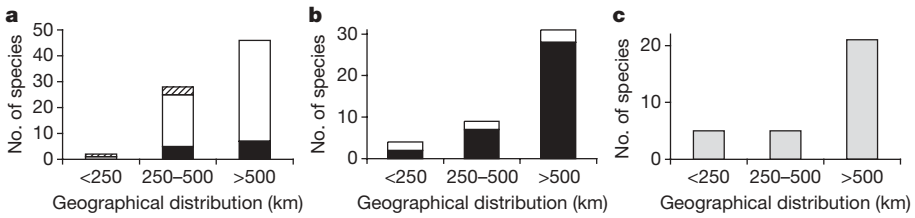

Figure 4 | Geographical distribution of caterpillar (a), ambrosia beetle (b) and fruitfly (c) species in Papua New Guinea lowland rainforests. Geographical distribution was quantified as the distance between the two most distant occurrences of the species. Species were classified as generalists, feeding on >1 genus (black); clade specialists, feeding on >1 species from a single genus (white); and monophagous, feeding on a single plant species (hatched). Host specificity of fruitfly species (grey) is unknown. Note that monophagous species could be recognized only in the Lepidoptera, as the ambrosia beetles were not reared from multiple congeneric plant species. Host specificity was not correlated with geographical distribution (caterpillars: Spearman r, $P > 0.25$; ambrosia beetles: Mann–Whitney U-test, $P > 0.20$). Only common herbivore species, listed in Supplementary Appendices 2–4, were analysed.

cordillera[26]. Tropical altitudinal gradients coincide with global maxima of plant species diversity[27] and the same is probably true for herbivorous insects. However, a large proportion of the world's tropical rainforest is situated in the more homogeneous lowland basins of major tropical rivers. For instance, the Sepik–Ramu and Fly–Strickland river systems comprise more than half of the lowland rainforest area in Papua New Guinea, and the two largest tropical forest areas, the Amazon and Congo basins, are also situated around large river systems[28]. Where relatively uniform altitude, climate and soil support a low beta diversity of vegetation[15,29], we argue that low beta diversity characterizes insect herbivores as well.

METHODS SUMMARY

The study included eight sites within a 500×150 km lowland area with continuous rainforest in the basin of the Sepik and Ramu rivers, and an additional site in the Papuan Peninsula (Fig. 1). Four large genera—*Ficus* (Moraceae), *Macaranga* (Euphorbiaceae), *Psychotria* (Rubiaceae) and *Syzygium* (Myrtaceae)—were the focus of the plant study. Each site hosted a floristic survey in a 5×5 km area and quantitative surveys in 50 plots, 20×20 m each.

The herbivore study included guilds feeding on leaves (caterpillars, Lepidoptera), wood (ambrosia beetles, Coleoptera) and fruit (fruitflies, Diptera). Caterpillars were sampled on 11–12 plant species from the four focal genera at each of eight study sites, surveying 1,500 m^2 of foliage per plant species[13,14]. Ambrosia beetles (Curculionidae: Scolytinae and Platypodinae) were sampled from four tree species—*Artocarpus altilis* (Moraceae), *Ficus nodosa* (Moraceae), *Leea indica* (Leeaceae) and *Nauclea orientalis* (Rubiaceae)—at three sites. Three individual trees from each study species were killed *in situ* at each site and after 20 days, standardized timber samples were hand-dissected for colonizing beetles. Dacine fruitflies (Tephritidae) were attracted to Steiner traps baited with lures (cuelure and methyl eugenol)[4]. Eight traps located in primary forest vegetation were operated for 6 weeks at each of four sites.

The similarity of plant and insect assemblages was quantified as the average proportion of species shared between sites, using the Sørensen index and its modification, the Chao–Sørensen index, which corrects for bias owing to incomplete sampling of rare species[30]. The probability $C(d)$ that two randomly selected individuals from different sites were conspecific was used as another measure of similarity[15]. Geographic distribution, measured as distance between the two most distant occurences, was estimated only for common species of insects. The effect of the Sepik River as a dispersal barrier was tested by comparing approximately equidistant assemblages of caterpillars feeding on the same and opposite sides of the river.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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1. Novotny, V. & Basset, Y. Host specificity of insect herbivores in tropical forests. *Proc. R. Soc. B* **272,** 1083–1090 (2005).
2. Lewinsohn, T. M., Novotny, V. & Basset, Y. Insects on plants: diversity of herbivore assemblages revisited. *Annu. Rev. Ecol. Evol. Syst.* **36,** 597–620 (2005).
3. Novotny, V. & Weiblen, G. D. From communities to continents: beta-diversity of herbivorous insects. *Annal. Zool. Fenn.* **42,** 463–475 (2005).
4. Novotny, V., Clarke, A. R., Drew, R. A. I., Balagawi, S. & Clifford, B. Host specialization and species richness of fruit flies (Diptera: Tephritidae) in a New Guinea rain forest. *J. Trop. Ecol.* **21,** 67–77 (2005).
5. Gaston, K. J. & Gauld, I. D. How many species of pimplines (Hymenoptera: Ichneumonidae) are there in Costa Rica? *J. Trop. Ecol.* **9,** 491–499 (1993).
6. Orr, A. G. & Haeuser, C. L. Temporal and spatial patterns of butterfly diversity in a lowland tropical rainforest. In *Tropical Rainforest Research — Current Issues* (eds Edwards, D. S., Booth W. E. & Choy, S.) 125–138 (Kluwer, Dordrecht, 1996).
7. Erwin, T. L. The biodiversity question: How many species of terrestrial arthropods are there? In *Forest Canopies* 2nd edn (eds Lowman, M. D. & Rinker, H. B.) 259–269 (Elsevier, Burlington, 2004).
8. Ruokolainen, K., Tuomisto, H., Vormisto, J. & Pitman, N. Two biases in estimating range sizes of Amazonian plant species. *J. Trop. Ecol.* **18,** 935–942 (2002).
9. Ødegaard, F. Host specificity, alpha- and beta-diversity of phytophagous beetles in two tropical forests in Panama. *Biodivers. Conserv.* **15,** 83–105 (2006).
10. Beck, J. & Chey, V. K. Beta-diversity of geometrid moths from northern Borneo: effects of habitat, time and space. *J. Anim. Ecol.* **76,** 230–237 (2007).
11. Brown, K. S. Jr. Geologic, evolutionary, and ecological bases of the diversification of Neotropical butterflies. In *Tropical Rainforests. Past, Present, and Future* (eds Bermingham, E., Dick, C. W. & Moritz, C.) 166–201 (Univ. Chicago Press, Chicago, 2005).
12. Howard, P. C. *et al.* Complementarity and the use of indicator groups for reserve selection in Uganda. *Nature* **394,** 472–474 (1998).
13. Miller, S. E., Novotny, V. & Basset, Y. Studies on New Guinea moths. 1. Introduction (Lepidoptera). *Proc. Entomol. Soc. Wash.* **105,** 1035–1043 (2003).
14. Novotny, V. *et al.* Local species richness of leaf-chewing insects feeding on woody plants from one hectare of a lowland rainforest. *Cons. Biol.* **18,** 227–237 (2004).
15. Condit, R. *et al.* Beta-diversity in tropical forest trees. *Science* **295,** 666–669 (2002).
16. Hubbell, S. P. *The Unified Neutral Theory of Biodiversity and Biogeography* (Princeton Univ. Press, Princeton, New Jersey, 2001).
17. Gascon, C. *et al.* Riverine barriers and the geographic distribution of Amazonian species. *Proc. Natl Acad. Sci. USA* **97,** 13672–13677 (2000).
18. Hall, J. P. W. & Harvey, D. J. The phylogeography of Amazonia revisited: New evidence from riodinid butterflies. *Evolution* **56,** 1489–1497 (2002).
19. Rosenzweig, M. L. *Species Diversity in Space and Time* (Cambridge Univ. Press, Cambridge, 1995).
20. Koleff, P., Lennon, J. J. & Gaston, K. J. Are there latitudinal gradients in species turnover? *Glob. Ecol. Biogeogr.* **12,** 483–498 (2003).
21. Höft, R. *Plants of New Guinea and the Solomon Islands. Dictionary of the Genera and Families of Flowering Plants and Ferns, Handbook no. 13.* (Wau Ecology Institute, Wau, 1992).
22. Novotny, V. *et al.* Response to comment on "Why are there so many species of herbivorous insects in tropical rainforests?". *Science* **315,** 1666 (2007).
23. Ødegaard, F. How many species of arthropods? Erwin's estimate revised. *Biol. J. Linn. Soc.* **71,** 583–597 (2000).
24. Novotny, V. *et al.* Low host specificity of herbivorous insects in a tropical forest. *Nature* **416,** 841–844 (2002).
25. Stork, N. E. How many species are there? *Biodiv. Cons.* **2,** 215–232 (1993).
26. Novotny, V. *et al.* An altitudinal comparison of caterpillar (Lepidoptera) assemblages on *Ficus* trees in Papua New Guinea. *J. Biogeogr.* **32,** 1303–1314 (2005).
27. Barthlott, W., Lauer, W. & Placke, A. Global distribution of species diversity in vascular plants: towards a world map of phytodiversity. *Erdkunde* **50,** 317–327 (1996).
28. Mittermeier, R. A. *et al.* Wilderness and biodiversity conservation. *Proc. Natl Acad. Sci. USA* **100,** 10309–10313 (2003).
29. Pitman, N. C. A., Terborgh, J., Silman, M. R. & Nuñez, V. P. Tree species distributions in an upper Amazonian forest. *Ecology* **80,** 2651–2661 (1999).
30. Chao, A., Chazdon, R. L., Colwell, R. K. & Shen, T. J. A new statistical approach for assessing similarity of species composition with incidence and abundance data. *Ecol. Lett.* **8,** 148–159 (2005).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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METHODS

Study sites. The study was located in the basin of the Sepik and Ramu rivers in northern New Guinea (Fig. 1a), within a 500 × 150 km area of lowland terrain with continuous rainforest and wetland vegetation. The area is populated by <10 people per km^2, has <1 km of roads per 100 km^2, and is bisected by a major tropical river, the Sepik. The river is up to 1 km wide while the accompanying belt of floodplain swamps, lakes and grasslands is up to 70 km wide[31] and represents the only large discontinuity in the rainforest ecosystem of the study area. The area is also among the most culturally diverse regions in the world, inhabited by populations speaking >200 different languages with even the most widespread ones only spoken across <5% of the region[32,33]. The study area is representative of lowland rainforests in Papua New Guinea as the Sepik–Ramu and Fly–Strickland river systems encompass the majority of these forests in this country.

We outlined an approximately equidistant grid of eight sites with an average distance of 160 km between neighbouring sites, and pair-wise distances ranging from 59 to 513 km (Fig. 1b). We also included one site in the Papuan Peninsula (Fig. 1a) to extend the range of between-site distances to 950 km. All sites were located in lowland rainforest <500 m above sea level with vegetation classified as mixed evergreen hill forest[34]. The climate at these sites is humid with a mean annual rainfall of 2,000–4,000 mm, with a moderate dry season from July to September (monthly mean rainfall ≤100 mm), and mean monthly air temperature ~26 °C. Soils are latosols[35]. The Madang area, approximately 20 × 30 km at the eastern boundary of our study area, including the Ohu study site and the town of Madang (Fig. 1a, b), was the focus of comparison with other study sites as it has a relatively well known insect fauna resulting from intensive study for >10 yr[4,13,14,36].

Each site consisted of a small village practicing subsistence agriculture in a matrix of primary and secondary rainforest (Fig. 1c). Four sites could be accessed by four-wheel-drive vehicle, others only by light aircraft. Each site was surveyed by a team including one researcher, four parataxonomists and ten locally hired assistants[37]. The surveys were conducted from December 2001 to July 2006 and included ~34 person-years under remote and challenging field conditions. Different sites were surveyed at different times of the year, avoiding the dry season and, in any case, insect seasonality in the area is low[38].

The study area was situated in a complex tectonic region at the convergence of two major plates: the Australian and Pacific. The northern New Guinea lowlands are a product of the gradual accretion of volcanic arc terranes to the central cordillera. The foothills of the central range that demarcate our study area in the south, and the Bewani and Torricelli ranges in the north, accreted to the existing landmass of New Guinea approximately 30–35 million years ago[39,40]. Furthermore, several terranes amalgamated to a single block, now forming the Papuan peninsula, which accreted about 15 million years ago. The last accretion event so far, involving the Adelbert and Finisterre blocks, was completed about 2 million years ago[41]. Most of the study area between the central and northern ranges was submerged from the Early Miocene until the Pliocene epoch[39,40]. Oceanic incursions across the northern lowlands during periods of elevated sea level continued until very recently, including a sea that stretched ~100 km inland and separated our Elem and Wamangu sites only 6,000 yr ago[42]. Alterations of climate and vegetation also occurred during the Holocene epoch. In particular, a mosaic of broadleaf open and closed forests covered the study area during a cooler and drier period about 17,000 yr ago[43].

Plants and insects. Large genera representing four plant families—*Ficus* (Moraceae), *Macaranga* (Euphorbiaceae), *Psychotria* (Rubiaceae) and *Syzygium* (Myrtaceae)—were the focus of the plant study. They are well represented in all stages of lowland rainforest succession[44] and together total at least 475 species in New Guinea[21]. Each site hosted a floristic survey of genera in a 5 × 5 km area and quantitative surveys of target plant species in 50 plots, 20 × 20 m each. Plots were divided evenly between primary and secondary forest types.

The study of herbivorous insects included guilds feeding on leaves (caterpillars, Lepidoptera), wood (ambrosia beetles, Coleoptera) and fruit (fruitflies, Diptera). Caterpillars (Lepidoptera) represent the most species-rich group of leaf-chewing insects in the study area[14]. Caterpillars were sampled during a 3 month survey staged at each study site from December 2001 to October 2005, except Popondetta where only ambrosia beetles and fruitflies were collected. Ohu hosted two consecutive surveys to assess the effect of sample size on our results. At each site, we collected caterpillars from 11–12 locally common plant species (4–5 *Ficus*, 3–5 *Macaranga*, 1–2 *Psychotria* and 1–2 *Syzygium* species per site) except at Ohu where we included 20 species that were sampled at one or more other sites (Supplementary Table 2). The selected species represented on average 40–86% of the basal area of each genus per site. These species are shrubs or small trees and represented <5% of the total basal area of the local woody vegetation (Supplementary Table 3). Target species included, as far as possible, a mix of those with widespread and limited geographical distribution across the study area.

Caterpillars were hand collected from approximately 1,500 m^2 of foliage per plant species per site. Each caterpillar was tested in a makeshift laboratory for feeding on the plant species from which it was collected and reared to an adult whenever possible (Fig. 1d). Only caterpillars that fed were retained for study. Species identifications were verified by dissection of genitalia, and when possible by reference to type specimens or in consultation with experts. Comparisons of mitochondrial cytochrome oxidase I (COI) DNA sequence divergence with morphology were used to identify polymorphic species including cases of sexual dimorphism[45,46]. Lepidopteran species are illustrated at http://www.entu.cas.cz/png/caterpillars. We recorded 74,184 caterpillars and 370 species feeding on the target plant species, including 25,437 individuals and 346 species reared to adults. Only information on reared adults was used in the analysis. We further characterized the Lepidoptera of the Madang area using additional data sets from Ohu and two nearby (<20 km) forest sites. These data comprised 56,002 caterpillars, including 19,011 reared to adults, from 580 species feeding on 94 plant species in 32 families, and 10,498 adults from 1,537 species collected at light (our own unpublished data and refs 13, 14).

Ambrosia beetles (Curculionidae: Scolytinae and Platypodinae) excavate tunnels in xylem of dead or moribund woody hosts, which they inoculate with symbiotic xylosaprophagous fungi, their sole source of food[47,48]. This feeding habit has evolved multiple times and allows ambrosia beetles to expand markedly their host ranges[49,50]. We sampled ambrosia beetles from four tree species: *Artocarpus altilis* (Park.) Fosb. (Moraceae), *Ficus nodosa* Teysm. & Binn. (Moraceae), *Leea indica* Merrill (Leeaceae) and *Nauclea orientalis* (L.) L. (Rubiaceae) at Ohu, Utai and Popondetta (Fig. 1). Three individual trees from each study species with diameters at breast height of 20–25 cm were killed at each site by ringing the bark and burning the base of the trunk at ground level. Trees were left standing for 20 days to attract ambrosia beetles. Standardized 1-m-long sections of trunk, 2 m of sections of branches 2–10 cm in diameter, and 2 m of sections of twigs <2 cm in diameter, were hand-dissected for colonizing beetles. Sampling was completed between February and July 2006. We obtained 12,751 beetles from 86 species. The ambrosia beetle fauna of the Madang area was further characterized by additional observations of ~70,000 individuals from at least 77 species reared from 15 tree species in 13 families (J.H., unpublished).

Dacine fruitflies (Tephritidae) feed as larvae on soft fleshy fruit. They are the most host-specific of the three guilds we studied, as most of the species are limited to a single plant genus or species[4]. They are endemic to subtropical and tropical rainforests from the Indian subcontinent across to Oceania, reaching their greatest diversity in New Guinea[51]. The adult fruitflies were attracted to Steiner traps baited with lures (cuelure and methyl eugenol) known to attract males of ~70% of the fruitfly species in Papua New Guinea[51]. Eight traps located in primary forest vegetation were operated for 6 weeks at each of four sites (Utai, Morox, Wanang and Popondetta, Fig. 1) from February to July 2006. We obtained 36,811 fruitflies from 46 species. The fruitfly fauna of the Madang area was further characterized by additional data from ~165,000 individuals and 69 species collected in traps[4,36,52] and from 168 species of plants supporting at least 29 fruitfly species[4].

Statistics. The geographical distribution of each species was measured by the number of sites, n, where it occurred and kilometres between the two most distant occurrences (5 km for species restricted to a single site). The sensitivity of these parameters to sample size was examined by randomization of species abundance among sites. It is necessary to compare our sampling effort to predictions of a null model because insufficient sampling may overestimate beta diversity[53].

The probability of observing a rare species (N< 3, 4 ... individuals) at n sites was estimated under the extreme case where beta diversity is zero. The probability that a particular site is occupied when N individuals are randomly distributed among n sites is $P = 1 - (1 - 1/n)^N$. We adhered to a threshold minimum abundance such that $P > 0.95$ for a randomly distributed species to be observed at all sites. The condition was satisfied by $N \geq 23$ individuals at eight sites sampled for caterpillars, $N \geq 8$ individuals at three sites sampled for ambrosia beetles, and $N \geq 11$ individuals at four sites sampled for fruitflies. We regarded these values of N as the minimum abundance for accurate detection of a species distribution in a particular herbivore guild. Only common species exceeding this minimum abundance were used to estimate geographical distribution and host specificity of insect herbivores. Seventy six of 370 Lepidoptera species, 31 of 46 fruitfly species, and 44 of 86 ambrosia beetle species met this threshold. The probability that N individuals are distributed so that any two particular sites, including the two most distant sites, are occupied is p^2 assuming no spatial autocorrelation of occurrences. The maximum possible geographical

span is of special interest because it was recorded for a surprisingly large number of herbivore species.

Host specificity of caterpillars and ambrosia beetles was assessed from feeding records combining all sites. Records of only a single individual feeding on a particular host species were excluded on the grounds that it is difficult to distinguish dubious records from genuine rarity. Monophagous species were defined as those feeding on a single plant species, clade specialists as those feeding on >1 species from a single genus, and generalists as those feeding on >1 genus. Monophagous species could not be separated from clade specialists in ambrosia beetles where only one plant species per genus was studied. No host information was available for fruitflies.

The Sørensen index, or the average proportion of species shared between two communities, was selected from a range of community similarity measures[53] because its modification, the Chao–Sørensen index, corrects for possible bias owing to incomplete sampling of rare species[30]. The original Sørensen index was applied to the plant records because abundance data were unavailable. Insect sampling included measures of species abundance, thus permitting calculation of the Chao–Sørensen index using EstimateS[54]. The mean Chao–Sørensen similarity between pairs of insect samples obtained from the same plant species during two successive surveys at Ohu approached unity as expected for identical assemblages (0.94 ± 0.015 for caterpillars sampled from 20 plant species and 0.94 ± 0.017 for ambrosia beetles sampled from three plant species), indicating that sample size was sufficient for inference of beta diversity. The significance of the correlation between geographical distance and community similarity was tested for caterpillars by the Mantel procedure. Only caterpillar assemblages from the 18 plant species studied at >3 surveys were included in this analysis. The similarity between lepidopteran communities was estimated from complete data on reared individuals, that is, including both rare and abundant species.

The effect of the Sepik River as a dispersal barrier was tested by comparing approximately equidistant assemblages of caterpillars feeding on the same and opposite sides of the river. The composition of the assemblage feeding on a particular plant species at a particular site (for example, Niksek, Fig. 1b) was compared with assemblages from nearly equidistant sites, one located on the same side of the river and the other on the opposite side (for example, Elem and Wamangu). Each insect sample from a particular plant species and a particular site was used only once in the analysis to preserve the independence of all comparisons. Nineteen matched pairs of samples from particular plant species were analysed, comparing sites 160 ± 12 km apart and separated by the river to sites 148 ± 6 km apart on the same side of the river.

The probability $C(d)$ that two randomly selected individuals from sites A and B were conspecific was calculated as $C(d) = \Sigma(n_{iA}/N_A)(n_{iB}/N_B)$ where n_i is the number of individuals from species i and N the total number of all individuals at a particular site. d denotes the distance between sites A and B.

31. Reiner, E. J. & Robbins, R. G. The Middle Sepik Plains, New Guinea: A physiographic study. *Geogr. Rev.* **54,** 20–44 (1964).
32. Wurm, S. A. & Hattori, S. *Language Atlas of the Pacific Area* (Australian Academy of the Humanities, Canberra, 1981).
33. Novotny, V. & Drozd, P. Size distribution of conspecific populations: peoples of New Guinea. *Proc. R. Soc. Lond. B* **267,** 947–952 (2000).
34. Paijmans, K. (ed.) *New Guinea Vegetation* (Australian National Univ. Press, Canberra, 1976).
35. Wood, A. W. The soils of New Guinea. In *Biogeography and Ecology of New Guinea* (ed. Gressitt, J. L.) 73–86 (W. Junk, The Hague, 1982).
36. Fletcher, B. S. Dacine fruit flies collected during the dry season in the lowland rainforest of Madang Province, Papua New Guinea (Diptera: Tephritidae). *Aust. J. Entomol.* **37,** 315–318 (1998).
37. Basset, Y. *et al.* Conservation and biological monitoring of tropical forests: the role of parataxonomists. *J. Appl. Ecol.* **41,** 163–174 (2004).
38. Novotny, V. *et al.* Predictably simple: communities of caterpillars (Lepidoptera) feeding on rainforest trees in Papua New Guinea. *Proc. R. Soc. Lond. B* **269,** 2337–2344 (2002).
39. Pigram, C. J. & Davies, H. L. Terranes and the accretion history of the New Guinea orogen. *J. Austral. Geol. Geophys.* **10,** 193–211 (1987).
40. Davies, H. L., Perembo, R. C. B., Winn, R. D. & KenGemar, P. Terranes of the New Guinea orogen. In *Proceedings of the Geology Exploration and Mining Conference, Madang* (ed. Hancock, G.) 61–66 (Australasian Institute of Mining and Metallurgy, Melbourne, 1997).
41. Abbott, L. D. Neogene tectonic reconstruction of the Adelbert-Finisterre-New Britain collision, northern Papua New Guinea. *J. S. E. Asian Earth Sci.* **11,** 33–51 (1995).
42. Swadling, P. Changing shorelines and cultural orientations in the Sepik-Ramu, Papua New Guinea: implications for Pacific prehistory. *World Archaeol.* **29,** 1–14 (1997).
43. Nix, H. A. & Kalma, J. D. Climate as a dominant control in the biogeography of northern Australia and New Guinea. In *Bridge and Barrier: the Natural and Cultural History of Torres Straight* (ed. Walker, D.) 61–92 (Australian National University, Canberra, 1972).
44. Leps, J., Novotny, V. & Basset, Y. Habitat and successional status of plants in relation to the communities of their leaf-chewing herbivores in Papua New Guinea. *J. Ecol.* **89,** 186–199 (2001).
45. Hebert, P. D., Penton, N. E. H., Burns, J. M., Janzen, D. H. & Hallwachs, W. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator. Proc. Natl Acad. Sci. USA* **101,** 14812–14817 (2004).
46. Hulcr, J. *et al.* DNA barcoding confirms polyphagy in a generalist moth, *Homona mermerodes* (Lepidoptera: Tortricidae). *Mol. Ecol. Notes* **7,** 549–557 (2007).
47. Beaver, R. A. Insect-fungus relationship in the bark and ambrosia beetles. In *Insect-Fungus Interactions* (eds Wilding, N., Collins, N. M., Hammond, P. M. & Webber, J. F.) 121–143 (Academic, London, 1989).
48. Mueller, U. G., Gerardo, N. M., Aanen, D. K., Six, D. L. & Schultz, T. R. The evolution of agriculture in insects. *Annu. Rev. Ecol. Syst.* **36,** 563–595 (2005).
49. Beaver, R. A. Host specificity of temperate and tropical animals. *Nature* **281,** 139–141 (1979).
50. Farrell, B. D. *et al.* The evolution of agriculture in beetles (Curculionidae: Scolytinae and Platypodinae). *Evolution* **55,** 2011–2027 (2001).
51. Drew, R. A. I. The tropical fruit flies (Diptera: Tephritidae: Dacinae) of the Australasian and Oceanian regions. *Mem. Queensl. Mus.* **26,** 1–521 (1989).
52. Clarke, A. R. *et al.* Distribution and biogeography of *Bactrocera* and *Dacus* species (Diptera: Tephritidae) in Papua New Guinea. *Aust. J. Entomol.* **43,** 148–156 (2004).
53. Koleff, P., Gaston, K. J. & Lennon, J. J. Measuring beta diversity for presence and absence data. *J. Anim. Ecol.* **72,** 367–382 (2003).
54. Colwell, R. K. EstimateS: Statistical Estimation of Species Richness and Shared Species from Samples. Version 7. 5 <http://purl.oclc.org/estimates> (2005).

BIODIVERSITY

World of insects

Nigel E. Stork

When it comes to understanding patterns of biodiversity, ours is a little-known planet. Large-scale sampling projects, as carried out in two investigations of insect diversity, show a way forward.

To a first approximation, all multicellular species on Earth are insects[1], and yet explanations for terrestrial biodiversity are largely based on birds, large mammals and plants. Studies of insect diversity by Novotny *et al.*[2] and Dyer *et al.*[3] (pages 692 and 696 of this issue) help to redress this imbalance, and provide an improved understanding of the distribution of global diversity.

Some 80–95% of insect species have yet to be collected, named and described, most of them living in the tropics. Even for the 850,000-plus species that have been named, we know little about how they are distributed or what they feed on[4]. Yet this information is essential for understanding the relationship between biodiversity and the functioning of global ecosystems. One reason is that a massive effort would be required to provide the field-based data for an analysis of patterns that might be applied generally at the global scale.

With the help of a team of locally trained parataxonomists, Novotny *et al.*[2] have compiled such a database of records for three groups of rainforest insects: those that feed on foliage (Fig. 1), wood and fruit. They show that there is a low rate of change in species composition, or 'β diversity', across 75,000 km^2 (an area equivalent to that of South Carolina or Ireland) of continuous lowland rainforest in Papua New Guinea. This contrasts with the previous evidence, as discussed by Novotny *et al.*, of high β diversity for insects in the forest canopy and with changes in β diversity with latitude, altitude and climatic gradients.

Novotny *et al.*[2] also show that insect species on host trees of the same genus, but separated by as much as 500 km, are remarkably similar, and that there do not seem to be barriers to their dispersal. The authors conclude that large, lowland areas of tropical forest, such as the Amazon and Congo, where there is low β diversity of vegetation, should also have low β diversity of insect herbivores.

In a previous paper, Novotny and colleagues[5] had compared their Papua New Guinea database of feeding records for the caterpillars of moths and butterflies, adult beetles and adult grasshoppers with similar records for taxa in temperate regions of Europe. They controlled for the relatedness of host trees, and concluded that the insect herbivores show similar levels of host specificity in both climatic regions.

In the second new paper discussed here, Dyer *et al.*[3] describe how they carried out an equivalent analysis in the New World and have come to a different conclusion. Their approach required examination of hundreds of thousands of host-specificity feeding records for butterfly and moth caterpillars, from as far back as 1936 and from areas ranging from Canada to Brazil. In contrast to Novotny and colleagues[5], they find that, on average, the number of tree species on which an insect species feeds is fewer in the tropics than in temperate parts of the New World. They suggest that higher specialization in the tropics might be because of more intense interactions between an insect and its food source, as might be caused by more distinct secondary chemicals in tropical plants than in temperate plants.

Dyer *et al.*[3] suggest that the difference between their results and those of Novotny *et al.*[5] may be due to true biological differences between the continents, or because Novotny *et al.* used only 8–14 focal host-tree species in the study as opposed to the large number of host trees in the Dyer *et al.* study. Other reasons may be in the way Dyer and colleagues' data sets were compiled, particularly differences between the older and much larger Canadian data set and the smaller, more recent data sets, and in the considerable differences in the sample sizes in the temperate and tropical data sets. Dyer and colleagues also suggest that there may be real differences in host specificity between the Americas, Europe and tropical Asia, but this seems unlikely. The question of which of these contrasting conclusions is correct will remain unresolved until further comparative studies take these sampling and geographical issues into account.

There has been an understandable bias towards the herbivorous insects in ecological studies[6], because insects have coevolved with the plants and trees on which they feed. Indeed, tree species richness may serve as the best proxy for overall biodiversity in tropical forests, as Terry Erwin inferred in his famous calculation[7] that raised estimates of tropical insect species tenfold to 30 million. Crucial suppositions he made were that each of the 50,000 tree species or groups of species in the world would have 165 host-specific beetle species, that beetles represent 40% of all insect species, and that the canopy is twice as rich in insect species as the ground, with the inference that species are stratum specific. His calculation implied that 84% of tropical insects are herbivores. The number of insect species that are specific to a particular tree species has since been carefully re-examined, however, and reduced by a factor of four or five[8].

But what of the insects that have less glamorous and obvious lifestyles than the herbivores: those that feed on dead and decaying material, or on the bacteria and fungi that break down organic material; or the predators and parasites

M. JANDA

Figure 1 | Foliage feeder. This magnificent caterpillar, the aptly named Hercules moth caterpillar, is one of some 500 species of insect herbivore investigated by Novotny *et al.*[2]. The authors conclude that there is a low rate of change of species composition (low β diversity) in the extensive lowland forests of the Sepik-Ramu basin in Papua New Guinea.

that feed on living plants and animals? The proportion of insect biodiversity that these 'feeding guilds' comprise is uncertain, but could be as high as 50–70%, and not 16% as Erwin proposed.

Looking beyond insects and setting aside microorganisms, what about fungi, other invertebrates and most marine life? These groups, too, are often poorly understood because of their taxonomic intractability or because they are so infrequently collected. The apparent rarity of many species in most samples of invertebrates and fungi is probably due to our low level of sampling rather than representing biological rarity. Making sense of such communities is almost impossible without the scale of sampling shown by Novotny and Dyer and their teams. Answers to such fundamental questions as how many species there are, how they are distributed, and how many are being lost through extinction will remain elusive without similar collaborative and large-scale enterprises. Of course, documenting how communities of organisms and their interactions change along ecological gradients is fundamentally more important than merely counting species.

So how much nearer are we to a model or group of models that might predict and explain the distribution of biodiversity on a global or even a regional scale? Roger Kitching[9] talked about "crafting the pieces of the diversity jigsaw puzzle", and these two new papers[2,3] help to identify a few more pieces of this puzzle. But we are still a long way from being able to explain the distribution of global biodiversity. Perhaps the nearest functional model is the mid-domain theory[10,11], which attempts to model the distribution of species and shows that species richness is greatest at the centre of a spatial, temporal or functional domain. But whether that theory can be expanded and modified remains to be seen. ■

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1. May, R. M. *Nature* **324,** 514–515 (1986).
2. Novotny, V. *et al. Nature* **448,** 692–695 (2007).
3. Dyer, L. A. *et al. Nature* **448,** 696–699 (2007).
4. Stork, N. E. in *The Living Planet in Crisis: Biodiversity Science and Policy* (eds Cracraft, J. & Grifo, F. T.) 3–32 (Columbia Univ. Press, New York, 1999).
5. Novotny, V. *et al. Science* **313,** 1115–1118 (2006).
6. Strong, D. R., Lawton, J. H. & Southwood, T. R. E. *Insects on Plants* (Blackwell Scientific, Oxford, 1984).
7. Erwin, T. L. *Coleopt. Bull.* **36,** 74–75 (1982).
8. Novotny, V. *et al. Nature* **416,** 841–844 (2002).
9. Kitching, R. L. *Science* **313,** 1055–1056 (2006).
10. Colwell, R. K & Hurtt, G. C. *Am. Nat.* **144,** 570–595 (1994).
11. http://viceroy.eeb.uconn.edu/RangeModel

CRYSTALLOGRAPHY

A down-to-Earth approach

John R. Helliwell and Naomi E. Chayen

In seeking out ideal conditions for growing protein crystals, solutions have increasingly been found in the low-gravity conditions of space. But answers might be lurking in fields closer to home.

Culturing high-quality protein crystals has, in the past decade, undergone a steady transformation from an art to science. That process has been assisted by exploiting the 'microgravity' conditions of space missions to lessen the fluid flows that disturb crystal growth on Earth's surface. As they describe in *Applied Physics Letters*, Heijna *et al.*[1] use an alternative approach: very strong, but inhomogeneous magnetic fields with which they establish a tunable gravity environment that, for crystal growth, recreates space on Earth.

Protein crystals are highly sought-after commodities for many basic studies in biochemistry and structural biology, and for structure-based drug design. The better the quality of a crystal, the better the structural information it yields. Microgravity conditions reduce buoyancy-driven turbulent flows in the 'mother liquor' from which a crystal emerges, and so are thought to promote crystal nucleation and ideal growth. In addition, such conditions remove the sedimentation effect of crystals heavier than the mother liquor. These near-perfect conditions have indeed been used to deliver bigger and better-formed protein crystals, to perform fundamental studies of crystal quality, and to produce homogeneous distributions of crystal sizes[2].

But experimentation in space has its disadvantages: restricted access, high costs (albeit mitigated by the small weight of the apparatus required) and political pressures, to name a few[3]. In addition, creating true microgravity conditions is difficult. Astronaut activity, for example, causes periods of gravity-like disturbance ('g-jitter')[4]. Although space has produced benchmark results, methods that are solely Earth-based have obvious attractions.

The inhomogeneous field (IHF) method harnessed by Heijna *et al.*[1] exploits a vertical magnetic-field gradient to create a force that counterbalances gravity. This approach is the basis of magnetic levitation techniques that have been used, among other things, to make frogs hover[5]. The precise values to which the field and its gradient must be tuned to negate gravity depend on the nature of the crystals' mother liquor and its density. By creating effective gravity conditions from g (normal gravity) down to $-0.15g$ (inverted gravity), the authors were able to slow down, halt and even reverse convection in the mother liquor (Fig. 1). An ingenious optical viewing set-up within the 32-mm-diameter borehole containing their magnetic field allowed them to view and monitor the growing crystal and its surrounding fluid directly.

This control of crystal-growth conditions is different from that brought about by microgravity: because the crystal and mother liquor respond to the magnetic field to different extents, convection (a property of the fluid) and sedimentation (a property of the crystals) are not eliminated simultaneously. This can be viewed in two ways. First, it is a limitation of the magnetic-field method. But second, it allows the experimental conditions 'convection-free' and 'sedimentation-free' to be separated out, and their relative importance in the growth of protein crystals to be evaluated. A caveat here is that, in an experiment to explore the accuracy of the settings in an IHF chamber used to grow inorganic crystals, residual fluid flows equivalent to around 0.5 $\mu m\ s^{-1}$ — about the same level as g-jitter in space — are found even when gravity is perfectly balanced out[6].

Besides the IHF approach, other methods, for example those using gels[7] and microfluidics, can provide the advantages of microgravity on Earth. Microfluidics, when combined with robotics for accurate and systematic screening of growth conditions, allows crystal-growth droplets as small as 10^{-11} m^3 (a hundredth of a cubic millimetre) to be accurately manipulated. In these small volumes, the problems of convection-driven fluid flows and sedimentation are scarcely relevant.

Of course, a crystal growing in such a small drop is also limited in size, but this presents little problem: modern synchrotron radiation facilities can analyse sample volumes of side just 20 μm (equivalent to about 10^{-14} m^3). An upgrade programme under way at the European Synchrotron Radiation Facility in Grenoble, France, to narrow the focus of its probing X-ray beam will lower this limit still further. In the upcoming new world of crystals numbering just a few thousand unit cells — 1,000 cells being 10 by 10 by 10 units — beams focused to 0.1 μm or less, equivalent to a probed volume of 10^{-21} m^3, will be required. Indeed, a challenge to the ingenuity of the engineers will be to incorporate the microfluidic and robotic stages necessary for the manipulation of such small volumes within the constrained volume of an IHF apparatus.

Protein crystallography with neutrons, which has the big advantage over X-rays of finding the positions of hydrogens (as deuterium atoms) even at relatively modest diffraction resolutions[8], uses larger protein crystals. But even here, improvements in