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

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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.

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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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CRYSTALLOGRAPHY

A down-to-Earth approach

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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

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