

## **Technical note**

Most explanations for why there are more species in one region of the world than another focus on either (1) the time it takes for species to be generated (a region with many species, such as the tropics, is ancient and undisturbed, whereas a region with few, such as the temperate, is younger and more disturbed) or (2) the rate at which species form or go extinct (for example, the tropics may be a "museum" where extinction is low, or a "cradle", where speciation is high compared to the temperate). A completely different explanation is based on the idea that some regions can accommodate more species than others. We provide evidence that this latter explanation is important for songbirds of the eastern Himalayas. Further, it appears that the formation of new species in the region is now low, much lower than it has been in the past: as new species have been produced it has become increasingly more difficult for additional ones to form. We argue that the east Himalayas has become filled up with bird species, because it is hard for new species to find a unique niche.

## **Popular summary**

The eastern Himalayas contains many species—it is one of the world's biodiversity hotspots. We show that these species formed many millions of years ago, adding further claim to the uniqueness and biological importance of this remote area.

An international team from India, the US, Germany and Sweden, led by Trevor Price of the University of Chicago, USA, and Dhananjai Mohan (formerly of the Wildlife Institute of India and now with the Indian Forest Service) obtained DNA sequences from all the Himalayan songbirds; the largest regional avifauna completely surveyed so far. As a group, the songbirds contain nearly half the world's bird species. More than 460 can be found in the Himalayas and about 360 of these can be found in a restricted area of the eastern Himalayas, which we believe makes it the location with the highest numbers of songbirds anywhere in the world (a similar sized area in North America or Europe typically contains less than 100.) We focused our research effort in the states of Bengal, Sikkim and the western part of Arunachal Pradesh. We found surprisingly large differences in DNA sequence between the species, despite some looking very similar.

We were able to translate the differences in DNA sequence to real time by building a tree of the relationships among all the species and calibrated the tree by using various bird fossils collected from elsewhere in the world. On average, it appears that each east Himalayan species has been separated from its next closest relative in the region for 6-7 million years. This is about the same length of time that humans and their closest relative, the chimpanzee, have been separated.

The songbirds are thought to have originated in the Australian region about 50 million years ago but our dates imply that a rapid cooling of the earth 34 million years ago triggered their spread into Asia. That cooling and their arrival in Asia appears to have set the stage for a spectacular adaptive radiation—the diversification of one species from an ancestral form to fill multiple ecological niches, producing those now familiar species such as swallows, larks, warblers, thrushes, wrens, nuthatches, finches and crows. All these different kinds arose within the first 10-12 million years of the start of the radiation.

Species that formed after that typically have much smaller ecological differences. Mostly, they involve living at different elevations, while continuing to eat roughly the same foods. Examples include the two flycatcher warblers and the two minivets illustrated in the attached figure. We speculate that climate change associated with the origin of the monsoon about 10 million years ago may have led to the appearance of additional habitats at higher elevations, stimulating the most recent round of species formation.

Through the last 6 million years, other species have formed elsewhere (e.g. in China and Siberia), but many have not spread into the east Himalayas. Our explanation is that it has become ever more difficult for these new species to find a unique way to make a living: the ecological space has become increasingly occupied. We set out to test this by studying the birds in the field. When we did this, we were greatly surprised to find that the place where most species are found is at about 2,000m elevation. This was surprising because we fully expected that the hot, wet subtropical forests in the states of Bengal, Assam and Arunachal Pradesh would contain the most species; one of the most famous patterns in ecology is that there are many more species in tropical than in temperate regions. Our further investigations showed that the 2,000m elevation has an abundance of small insects during the breeding season, and a multitude of small bird species feeding on these insects. We are now trying to understand why there should be more insects at this elevation, but this kind of association (between food availability and numbers of species) is just what one would expect to see if the eastern Himalayas is full with bird species.

Sequencing all the songbirds of the Himalayas was not straightforward. We obtained many sequences from ancient specimens in European and North American museums, which required special techniques to extract the DNA. Other sequences were taken from tail feathers that were collected from birds we captured and released during the course of our field studies. From the feather base, we extracted the DNA and sequenced it at the Wildlife Institute of India. In this way, we were able to learn about several extremely rare species, some of which are not in any museum collection. For example, the Bugun liocichla, a beautiful bird only discovered 10 years ago by Ramana Athreya of the Indian Institute of Science Education and Research in Pune, is still only known from a few pairs breeding at the edge of the Eaglenest Wildlife Sanctuary in Arunachal Pradesh. Dhananjai Mohan, Mousumi Ghosh (a graduate student at the Wildlife Institute of India) and University of Chicago post-doctoral researcher Thomas Tietze captured a bird in 2010, creating so much excitement that Tietze almost smashed his camera in the process. Tietze then sequenced the DNA and found it was separated by >3 million years from its closest relative living in the mountains of central China. This places the divergence of the two species from before the beginning of the ice ages, emphasizing the uniqueness of the Bugun liocichla, and also the importance of such genetic studies in conservation efforts.

The past few million years have seen great fluctuations in climate, exemplified by repeated incursions of ice sheets. In the eastern Himalayas, an amazing community of birds and other species seems to have persisted through multiple challenges posed by climate changes over this time, providing a refuge, a place from which species could expand out during periods when the climate improved; and into which they could retreat when the climate deteriorated. We consider northeast India to be India's "great barrier

reef", albeit an inverted and terrestrial version, full of beautiful species of birds, reptiles and mammals. We now know that, for the birds at least, this place contains exceptional genetic diversity as well. We hope our results on the birds will encourage further research on other groups, such as the reptiles and mammals, and also encourage more people to visit this area.

Some special birds of the east Himalayas: Clockwise from top left: A) Two similar species of flycatcher warbler, estimated to be separated from each other for more than 6 million years. B) Two similar species of minivet, separated for perhaps 4 million years. C) The spotted wren babbler, which has no close relative at all, it separated from other present-day species perhaps 25 million years ago. D) The Bugun liocichla is probably separated from its closest relative in China by more than 3 million years. Higher resolution pictures of these are available.

