For the first time: a Nobel Prize for Evolutionary Research

Although evolution forms the conceptual framework within which we organize and interpret biological knowledge at multiple levels of structural organization, ranging from molecules to ecosystems, the Nobel Prize in the biological sciences is actually awarded for 'Medicine or Physiology', rendering it almost impossible for evolutionary research, however important or consequential, to be recognized. Between 1901 and 2021, the Nobel Prizes and the Sveriges Riksbank Prize in Economic Sciences in Memory of Alfred Nobel, have been awarded 609 times to 975 people (943 distinct individuals). Thus far, the only exception made by the Nobel Committee to recognize work not directly related to medicine or physiology was in 1973, when Nikolaas Tinbergen, Konrad Lorenz and Karl von Frisch shared the Nobel prize for their work on understanding how individual and social patterns of behaviour in animals are generated, organized and elicited. As evolutionary biologists, we have been pleasantly surprised this year to see the 2022 Nobel Prize in Medicine or Physiology being awarded to Svante Pääbo for his important contributions to our understanding of the early stages of human evolution. The intertwining of genetics and evolution in Pääbo's work mirrors the meshing together of heredity and evolution since Darwin. During his five years of voyaging around the world aboard the HMS Beagle, Darwin observed the ubiquity of, and biogeographical patterns in, the variability and diversity of natural populations of species. He attempted to explain those patterns through the principle of natural selection, developed by analogy to breeding, bolstering his arguments with evidence from comparative anatomy, embryology, and palaeontology. Darwin thus suggested that all extant life-forms had descended from different life-forms that lived in the past, a notion he termed 'Descent with Modification', with selection providing an explanation for the accumulation of adaptive modifications in lineages.

In the absence of any knowledge about the mechanisms governing the generation of variations and their inheritance, Darwin assumed that variations arose ubiquitously, and were heritable to a reasonably strong degree, resulting in significant parent–offspring similarity with regard to such variations. With the rediscovery of Mendel's work on heredity in 1900, it became important to establish whether the principles of Mendelian heredity were conducive to the effective operation of the proposed mechanism of selection in mediating adaptive evolutionary change, especially given the past doubts about whether selection could bring about evolutionary change in the face of heredity, conceived by Galton and others as constituting a conservative force tending to move the next generation back towards the population mean. By 1930, the issue was largely resolved: Mendelian inheritance *per se* was shown not to alter the genetic composition of populations, at least under random mating, to be entirely compatible with all the statistical results on correlations between relatives, and to permit adaptive change in the genetic composition of populations under selection (a detailed account of this phase of the welding together of genetics and evolution can be found in Gayon[1], and a more accessible account in Vidya[2]). The next couple of decades saw the bringing together of Mendelian genetics, the principle of natural selection, cytogenetics, systematics, and palaeontology into what has been termed the 'Modern Synthesis'[3]. It is worth noting that, till this point in time, the genetic material had not yet been identified as being DNA. Following the discovery of DNA structure, and the elucidation of the mechanisms of its expression, evolutionary genetics was further enriched with a more mechanistic understanding of the modulation of gene mutation and expression. This, in turn, led to a greater appreciation of stochastic changes in DNA sequence at neutral loci, leading to the development of phylogenetic techniques for inferring interrelationships between extant species, and how lineages may have branched in the past.

After the development of sequencing techniques, it became possible to sequence entire genomes, first of bacteria or viruses, and later, the much larger genomes of metazoans. Ultimately, the Human Nuclear Genome Project was launched in 1990 by a large international consortium with the objective of sequencing and annotating a full haploid nuclear genome to generate a human reference map. The findings were published in 2001, describing the sequence of the human nuclear genome. This was followed by mapping the complex regions of the genome[4–6]. The technology and expertise of genome sequencing was standardized so much that 'Robots' are capable of achieving it with precision, and today voluminous data is available. With the success stories of genome biology of a number of animals and plants, microbes (including Archean species), new challenges as well as novel questions have come up. With these developments, new disciplines such as functional genomics and comparative genomics have taken shape. What is often not appreciated enough, especially in India, is that although the generation of genome sequences requires the application of molecular genetics and bioinformatic tools, the real interpretation and usefulness of genome sequence data is for addressing evolutionary questions. Conversely, even the use of genomics data for biomedical research depends heavily on theory of evolution for proper inferences to be drawn.

One of the offshoots of the developments and diversification in genetics and genomics after the elucidation of DNA structure and the advent of DNA sequencing methods has been the field of palaeogenomics. The discipline of palaeontology, with the fossil record serving as 'documentation' of evolution, confirmed the existence of now-extinct species and in a few cases, captured potential transitional forms between extinct and contemporary species. A palaeogenome is the genome of an extinct organism; for example, the genome of a dinosaur, a Neanderthal, or an extinct plant[7]. Studying palaeogenomes, and comparing them with genomes of extant relatives or descendants can greatly clarify our understanding of evolutionary events in any lineage. As far back as 1993 (ref. 8), the Department of Science and Technology of the Government of India organized a 'brainstorming session' on 'Paleobiochemistry' at Punjab University, Chandigarh. In that session, the importance of studies on molecular palaeontology and molecular archaeology was discussed. Eventually, the development of techniques for reliably extracting DNA from ancient remains, coupled with better and cheaper sequencing technologies, has added the capability to read the sequence of genomes from extinct organisms to the tool-kit of molecular palaeontologists.

Svante Pääbo, the Nobel laureate of this year, and his group worked for over two decades and succeeded in establishing a protocol to reliably extract and sequence the genome from ancient remains. The story of the work of this group reflects the commitment, dedication, and perseverance

of scientists unperturbed by failures, as well as collaboration and cooperation among different laboratories.

Pääbo and his collaborators initially focused on remains of the Neanderthals (*Homo neanderthalensis*), a species of hominin that went extinct about 30,000 years ago, with whom we (*H. sapiens*) shared a common ancestor in *H. heidelbergensis,* and also coexisted for about 70,000 years. Despite many technical and procedural difficulties, they finally perfected the techniques to extract and amplify uncontaminated Neanderthal DNA and reconstruct the nucleotide sequence of (almost) the entire genome in 2010 (ref. 9). Access to the Neanderthal genome sequence permitted a better resolution of some of the speculative controversies that existed about the period of human–Neanderthal coexistence, and the eventual extinction of the latter species. This year's Nobel Prize in Physiology or Medicine has been awarded for these pioneering studies, using precious snippets of DNA found in fossils that are tens of thousands of years old, that have shed new light on many aspects of human evolutionary history over the past 70,000 years or so[10]. At every stage in this work, methodological improvements were made by adopting emerging sequencing technologies, new algorithms for analysing and mapping the data, taking extraordinary care to avoid contamination, and carefully comparing the Neanderthal data with the sequences from *H. sapiens*, chimpanzee, and other related species.

To begin with, Pääbo concentrated on the mitochondrial genome of Neanderthal fossils found in Germany; the work was published in 1997 (ref. 11). The group then extended their investigations to the nuclear genome of Neanderthal fossils, testing more than 70 Neanderthal bone and tooth samples from different sites in Europe and western Asia. One bone from Vindija Cave, Croatia, stood out, in that >90% of the mtDNA segments were of Neanderthal origin. Subsequently, Pääbo studied genomic DNA of additional specimens from the Vindija Cave, from the Mezmaiskaya cave in the Caucasus, and the El Sidrón cave in Spain. Varied expertise was now needed for analysis and interpretation of the data, including inputs from population geneticists, and a consortium of around 50 scientists was established. DNA from these remains resulted in sequence information encompassing more than 4 billion nucleotides, which were then mapped and analysed. In 2010, Pääbo and co-workers published a draft Neanderthal nuclear genome sequence. Five early *H. sapiens* genomes were also sequenced and compared with a collection of 2051 present-day human and 59 chimpanzee sequences to aid in comparative data analyses[9]. Neanderthals from across a great part of their range in western Eurasia seemed more or less equally related to present-day humans, with the average divergence of Neanderthal and present-day human nuclear DNA sequences estimated to be about 825,000 years. The Neanderthal sequence was shown to fall outside the variation observed in present-day sequences of *H. sapiens* of European, African, Asian, Native American, and Australian/Oceanic origin. The question of whether interbreeding had occurred between Neanderthals and anatomically modern humans could now be investigated directly, by nuclear genome sequence analyses, leading to the finding that Neanderthals and *H. sapiens* had indeed interbred, and that 1–4% of the genome of modern humans of European or Asian descent can be traced back to the Neanderthals.

In 2008, the distal phalanx of the fifth manual digit of a juvenile hominin was excavated in Denisova Cave in the Altai mountains, Russia, in a stratum dated to 48,000 to 30,000 years ago. From this finger bone, Pääbo's group extracted DNA, which was found to be exceptionally well preserved. This was sequenced and assembled to a complete mtDNA (mitochondrial DNA) sequence[12]. Whereas Neanderthal mtDNA differs from that of *H. sapiens* at an average of 202 nucleotide positions, the sample from the finger bone specimen differed at an average of 385 positions, and from the chimpanzee mtDNA sequence at 1,462 positions. Thus, the mtDNA from the unknown hominin was considerably more divergent from present day humans than from Neanderthal mtDNA. Pääbo's team went on to sequence the nuclear genome from DNA extracted from the Denisova finger bone. They mapped the sequences to the human and chimpanzee reference genomes, as well as to the inferred ancestral genome of these species, generating a Denisova genome sequence with about 1.9-fold coverage. The studies showed that Denisovans were a group of extinct hominins that were more closely related to Neanderthals than to modern humans, and that they lived in the Siberian cave more than 30,000 years ago. Pääbo's group had discovered an entirely new hominin, distinct from Neanderthals and *H. sapiens* and called it as Denisovan, the region from where the bones were collected. Subsequently, a direct descendant of two different groups of early humans was found in Russia. This ancient hominin hybrid[13] called Denny had acquired one set of chromosomes from a Neanderthal and the other from a Denisovan, and it is believed by Pääbo that interbreeding between Neanderthals and Denisovans was common[13]. The genomic data, thus, provide evidence for hybridization among hominins, that is, between Neanderthals and Denisovans, as well as between both Neanderthals/Denisovans and early *H. sapiens.* Hybridization was recognized as a potentially important evolutionary factor by Darwin in his book, 'Origin of species'. Ranganath[14] while commenting on Darwin's finches in the Galapagos island as a goldmine for evolutionary biologists has discussed the occurrence of inbreeding across different groups of animals and treated 'hybridization' as an evolutionary catalyst in promoting diversification.

The members of the Nobel Committee namely Gunilla Karlsson Hedestam and Anna Wedell (Advanced Information 2022) have expressed that Svante Pääbo's work further established that *Homo sapiens* had mixed with Neanderthals and Denisovans during periods of co-existence, resulting in introgression of archaic DNA in present-day humans. Striking examples of archaic gene variants that influence the physiology of present-day humans have already been demonstrated in a research field that is now highly dynamic. Through his groundbreaking discoveries, Pääbo opened a new window to our evolutionary past, revealing an unexpected complexity in the evolution and ad-mixture of ancient hominins, as well as providing the basis for an improved understanding of genetic features that make us uniquely human. Segments that derive from Neanderthals can be found in most regions of the human genome, albeit at different frequencies in different parts of the genome. There is now strong evidence that some of these signals represent archaic haplotypes derived from introgression events. A clear example is the Denisova-derived version of the hypoxia pathway gene, EPAS1, which confers a genetic advantage to survival at high altitude and is found in present-day Tibetans. Another intriguing example of introgression is a cluster of genes encoding three Toll-like receptors, *TLR6-TLR1-TLR10*, known to be involved in microbial recognition and allergic reactions. Later work by Zeberg and Pääbo[15] indicated that some of our immunity-related genes may be of Neanderthal origin. A haplotype on human chromosome 12, which

is associated with a ~22% reduction in relative risk of becoming severely ill with COVID-19 when infected by SARS-CoV-2, appears to have been inherited by modern humans from the Neanderthals. This haplotype is present at substantial frequencies in humans in all regions of the world outside Africa. The genomic region where this haplotype occurs encodes proteins that are important during infections with RNA viruses. In a recent publication from Pääbo's group[16], the likelihood of a longer metaphase duration and fewer chromosome segregation errors in modern human than Neanderthal brain development states has been reported. In the time since the ancestors of modern humans separated from those of Neanderthals, around 100 amino acid substitutions seem to have spread to essentially all modern humans. The biological significance of these changes is largely unknown. Pääbo's team have shown all six such amino acid substitutions in three proteins known to have key roles in kinetochore function and chromosome segregation and to be highly expressed in the stem cells of the developing neocortex. When they introduced these modern human-specific substitutions in mice, three substitutions in two of these proteins, KIF18a and KNL1, caused metaphase prolongation and fewer chromosome segregation errors in apical progenitors of the developing neocortex. Conversely, the ancestral substitutions caused shorter metaphase duration and more chromosome segregation errors in human brain organoids, similar to what we find in chimpanzee organoids. These results imply that the fidelity of chromosome segregation during neocortex development likely improved in modern humans after their divergence from Neanderthals.

In 2010, Rasmussen *et al.*[17] published details of the first human palaeogenome, which they had isolated from a remarkably well-preserved Saqqaq palaeo-eskimo hair sample. The authors were able to recover 79% of the genome, identifying 350 000 SNPs. Analyses of these SNPs allowed Rasmussen *et al.*[15] to corroborate the mitochondrial relationship of the Saqqaq individual to modern human populations, to identify his blood type (A+), and also to estimate his eye colour, based on a variant of the HERC2-OCA2 locus that is strongly associated with brown eyes. They also identified 12 SNPs that have been associated with adaptation to a cold climate by influencing metabolism and body mass index. Thus, archaic gene flow into *Homo sapiens* influences human physiology has occurred, offering exciting possibilities to elucidate how specific gene variants modulate biological processes at the molecular level. Therefore, it may be inferred that the genes themselves may be older than the species that bear them.

Overall, the discoveries of Pääbo and his group have had a profound impact on our understanding of early human evolutionary history, and have galvanized further research in the area. For example, we now know that at least two distinct hominin groups, Neanderthals and Denisovans, inhabited Eurasia when anatomically modern humans (*H. sapiens*) emerged from Africa to spread all over the world, and that our ancestors interbred with them, such that we now carry some amount of both Neanderthal and Denisovan genes in our genomes. Palaeogenomic analyses, thus, can provide insights as to when and by what means different traits might have evolved, and how extinct species are related to living species and populations. It is now possible to detect evolutionary events, ancient population migrations and interrelationships, and the evolutionary histories of extinct plant, animal and *Homo* species.

We are particularly happy that this year's Nobel Prize for Medicine or Physiology has recognized an aspect of evolutionary biology research. In India, evolutionary biology was traditionally neglected, but that situation has changed a lot in the last 25 years. We now have a growing, very active, and well recognized evolutionary biology community in our country, and the number of publications regularly appearing in the top journals on evolution from India has increased very rapidly over the last two decades. Indeed, India has also contributed to palaeogenomics research, most notably through the work of K. Thangaraj at the CSIR-CCMB, Hyderabad, as well as others. In experimental evolution, too, India is exceptionally well represented, accounting for almost half of the research groups in the world. In 2018, the Indian Society of Evolutionary Biologists (https://home.evolutionindia.org) was established, under the Presidentship of Raghavendra Gadagkar, one of our most distinguished evolutionary biologists and an internationally recognized authority on social evolution in hymenopteran insects such as wasps, ants and bees. The society has been active in organizing national and international conferences, as well as outreach events aimed at students. We hope that this year's Nobel Prize to Svante Pääbo will encourage more Indian students to take up evolutionary biology as their research career.

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