The genetic legacy of the Quaternary ice ages

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Global climate has fluctuated greatly during the past three million years, leading to the recent major ice ages. An inescapable consequence for most living organisms is great changes in their distribution, which are expressed differently in boreal, temperate and tropical zones. Such range changes can be expected to have genetic consequences, and the advent of DNA technology provides most suitable markers to examine these. Several good data sets are now available, which provide tests of expectations, insights into species colonization and unexpected genetic subdivision and mixture of species. The genetic structure of human populations may be viewed in the same context. The present genetic structure of populations, species and communities has been mainly formed by Quaternary ice ages, and genetic, fossil and physical data combined can greatly help our understanding of how organisms were so affected.

The study of palaeoclimates is a particularly active research field that is producing much data and increasingly coherent explanations. The Earth's climate became cooler through the Tertiary (65 million years (Myr)) with frequent oscillations that increased in amplitude and lead to the series of major ice ages of the Quaternary (2.4 Myr to the present). The evidence for such global fluctuations in climate comes particularly from cores of the sea bed, lake bottoms and ice sheets, which are analysed for carbon and oxygen isotopes, radiolarian species, pollen types and other biological and physical signatures[1,2].

Ice ages and species distributions

While the Antarctic ice cap grew from the Oligocene (35 Myr), the Arctic ice cap became established about 2.4 Myr ago, the beginning of the Quaternary. From then until 0.9 Myr ago, the ice sheets advanced and receded with a roughly 41,000-yr (41-kyr) cycle; thereafter they have followed a 100-kyr cycle and become increasingly dramatic. Such periodicity suggests a controlling mechanism, and the Croll–Milankovitch theory proposes that the regular variations in the Earth's orbit around the Sun are the pacemakers of the ice-age cycles[1,2]. The main orbital eccentricity has a 100-kyr cycle, variation in the Earth's axial tilt has a 41-kyr cycle, and precession due to the Earth's axial wobble has a 19–23-kyr cycle; these all modify the insolation of the Earth and the energy it receives. Much energy is transported by the oceanic circulation system, and the interaction of orbital variation and currents leads to significant climate changes[2,3].

It is now possible to extract ice cores of ~2 km in length and analyse the annually layered snow for entrapped gases, isotopes, acidity, dust and pollen. Some recent cores sample ice over 400 kyr (ref. 4), but most go back ~125 kyr from the present to the previous (Eemian) interglacial. Long pollen cores stretching back to 400 kyr are also becoming available[5]. Along with other measures, these are providing a detailed picture of the last glacial cycle and glimpses of the preceding ones (Fig. 1). The Greenland (Arctic) and Vostok (Antarctic) ice cores are particularly informative, offering fine temporal resolution and continuity[2]. This has revealed surprising oscillations of climate on a millennial scale within the main 100-kyr cycle. The Greenland Ice Core Project (GRIP) identifies some 24 interstadials through the last ice age with average temperature rising rapidly by ~7 °C over just decades. Further ice and sediment cores from around the world are demonstrating the global scale of these major climatic events[6,7]. As more long cores of ice, sediment and pollen become available, it will be possible to produce a synthesis of the effects of these rapid climatic switches on plant and animal life worldwide[5,8].

These severe climatic oscillations produced great changes in species distributions, and these have been described in some detail, particularly from the fossil records of pollen and beetles in Europe and North America[1,9]. Species went extinct over large parts of their range, some dispersed to new locations, some survived in refugia and then expanded again, and this must have occurred repeatedly.

During major glaciations the polar ice sheets spread considerably, and temperature, marine and vegetation zones were compressed towards the Equator[2]. Mountain blocks like the Alps, Andes, Rockies and Yakutsk ranges also had considerable glaciation, so that the large volume of accumulated ice reduced sea levels by about 120 m (ref. 10). This produced land bridges in several parts of the world (Fig. 1). In warmer parts species descended from mountains. Tropical rainforest was restricted and dissected, and there was extension of deserts and savannah[11]. It is apparent that these major climatic shifts were felt differently across the globe owing to regional differences in landform, ocean currents and latitude. Furthermore, species responded individually, and their range changes were particular to local geography and climate.

Genetic consequences

In Europe and North America, an extensive network of pollen cores tells us that species now inhabiting boreal and temperate regions had their ice-age refugia south of the ice and permafrost. Post-glacial expansion into new territory was previously suggested to be important in the geographic distribution of population and species genomes[12]. It was remarkably rapid for many species, and the suddenness of the large climatic shifts recorded in the recent ice cores helps explain this. Populations at the northern limits of the refugial range would have expanded into often large areas of suitable territory. This leading edge expansion would probably be by long-distance dispersers that set up colonies and rapidly expanded to fill the area before others arrived. This would be repeated many times over a long colonizing route, and these founding events would lead to loss of alleles and homozygosity[13]. Modelling and simulations of such range expansion show that leptokurtic dispersal produces large areas of homozygosity as compared with normal or stepping-stone modes, and these homogeneous areas persist in space and increase with time[14]. Secondary oscillations within the main expansion increase the effect. Rapid colonization by this leading edge model in any part of the world should produce areas with reduced genomic variability.

Where the postglacially colonized regions are known from the fossil record (in Europe and North America), a growing number of studies show them to have lower genetic diversity[15], and a fine

analysis of 41 North American fish species demonstrates this clearly[16]. These studies also reveal that equivalent genomes occupy larger ranges in these colonized areas, as produced in simulation modelling[14].

An interesting corollary of this leading edge expansion is that when populations have filled the space it is much more difficult for those behind them to advance, because they must disperse and reproduce logistically, and not exponentially like the original scattered colonists[13]. This high-density barrier will mean that boundaries between expanded edge and blocked interior genomes will tend to persist for some time and be seen in present surveys[15,16]. It is also likely that during a range change a retreating rear edge will suffer shrinkage, dissection and extinction, so that the last surviving population should be severely bottlenecked. This could be in the vanguard of the recolonization.

With expansion of range there will also be selection and adaptation to different environments and new neighbours[13,15]. For example, the reproductive biology of many plants is suited to different light and temperature regimes across their present range from south to north[17], which has been produced with postglacial colonization. The same is true of insects, and particularly nice demonstrations are seen in recent invasions with accompanying modifications of life history, such as that of the USA by the cornborer moth *Ostrinia nubialis*[18]. Theoretical and experimental work in flies has shown an increase in genetic variance after severe bottlenecks, probably due to the reassortment of epistatic interactions[19]. In the pitcher-plant mosquito, *Wyeomyia smithii*, which ranges from Florida to Labrador, a reduction in allozyme diversity and heterozygosity in the postglacial expansion range in the north is reflected in an increase in additive genetic variance for development time and photoperiod response[20]. The genetic architecture has been remodelled by the dynamics of colonization. Such reorganization during colonization would compound divergence between population genomes. With repeated climatic oscillations and range changes, a population may pass through many such adaptations and reorganizations and its genome structure could diverge considerably.

The rapid expansion across large parts of Europe and North America was not followed by all species, and was probably much slower in some other regions, particularly the tropics. This would maintain a larger effective population size and retain much more genetic diversity. Various parts of the same species range may have been colonized at different rates owing to physical barriers or prior inhabitants, producing distinct genetic structures. Mountain blocks in southern temperate regions and the tropics would allow slower altitudinal shifts in range, tending to retain allelic diversity. Their varied topography also tends to subdivide the species into populations that may evolve independently with only occasional gene flow, perhaps only every glaciation or interstadial. It would seem that the interpretation of present genetic structure needs to consider such interaction of biology, geography and climatic shifts.

Suitable DNA markers

Modern DNA technology allows genetic diversity to be measured as single-base changes in many individuals, and a variety of sequences have been used (Table 1). As the Quaternary is 2.4 Myr old, most DNA sequences will diverge little over the ice ages, and few new mutations will distinguish postglacial haplotypes. Consequently, differences in such markers between postglacial population genotypes are likely to be due to the sorting of mutations that have mostly originated in the more distant past. Some regions in the D-loop of human mitochondrial (mt) DNA have been shown to be hypervariable, providing useful distinguishing mutations. Some care is called for in the interpretation of patterns[21,22], and there has been considerable development of techniques for probing population history. Population bottlenecks are inferred by mismatch comparisons[23], aspects of demography and phylogeography can be discerned using nested clade analysis[21], and spanning haplotype networks can reveal both demographic and geographic history[24]. These are now being applied effectively to human populations.

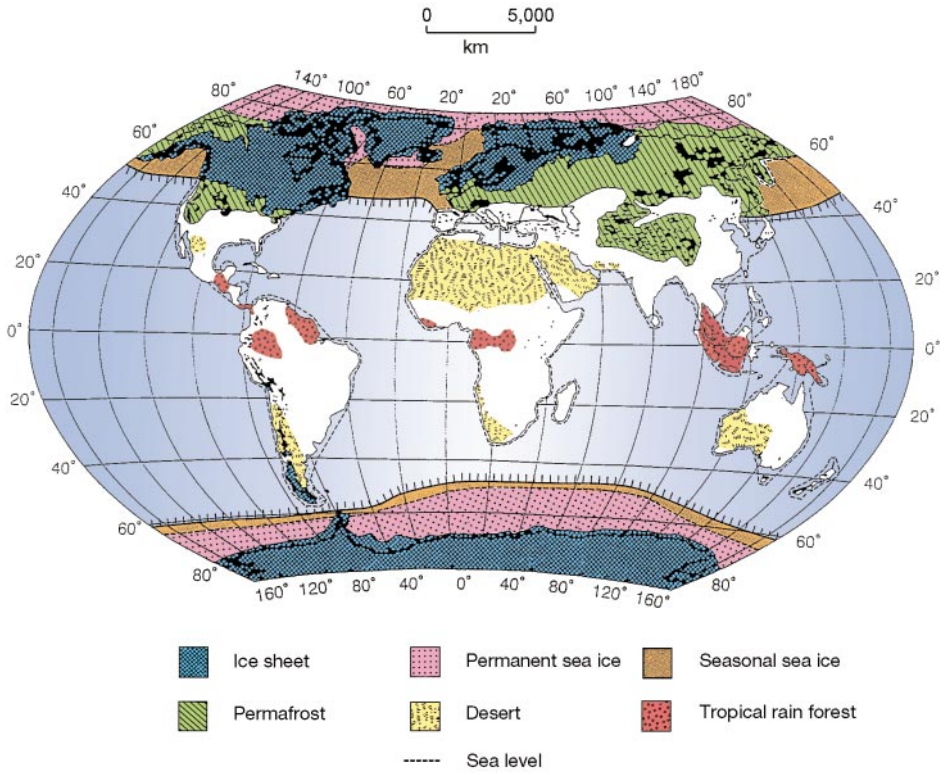

Figure 1 The maximum extent of ice and permafrost at the end of the last ice age 20,000 yr BP. The lowered sea level, large deserts and main blocks of tropical forest are indicated. Modified with permission from ref. 2.

European case studies

Research accumulated before the discovery of polymerase chain reaction (PCR) showed that the distribution of many species are subdivided by narrow hybrid zones; these were produced by the meeting of two diverged genomes as they expanded their ranges from separate glacial refugia[12]. With methods such as PCR, it is now possible using DNA similarity to show from which refugia particular genomes emerged to cover their present distribution. There are now a few species that have been studied using suitably variable and discriminating sequences in a large enough number of samples from across their range[15,25]. Wide-range coverage is important to produce a full phylogeography and because the effects of leading and rear edge, refugia and altitudinal shifts are expected to be different. Twelve such cases have been collated[22] in which likely postglacial colonization routes from their refugia across Europe can be deduced. Although each species colonization has its own colonization history and geographic pattern of genomes, three broad patterns are evident—the grasshopper, the hedgehog and the bear—which may serve as paradigms.

Chorthippus parallelus, the common meadow grasshopper of Europe was analysed using a unique non-coding nuclear sequence[26], which revealed that its species genome was divided into at least five main geographic regions. Across all northern Europe the haplotypes showed little diversity and were similar to those in the Balkans, clearly indicating a postglacial expansion from a Balkan refugium. Turkey, Greece, Italy and Spain each contain a large portion of unique haplotypes, which shows that they contained refugia and that these populations were limited to these southern regions. Hybrid zones have been described between the Spanish and French genomes and the Italian and French/Austrian genomes along the Pyrenees and Alps where the expanding genomes met[27], and may also occur where other genomes meet in southern Europe. Pollen analysis shows that the vegetation that currently supports these grasshoppers was restricted to parts of southern Europe during the last glacial maximum, and notably demonstrated rapid postglacial advance in the east (Fig. 2).

The alder, beech and crested newt have genome patterns that are broadly similar to that of the grasshopper, with their deduced colonizations dominated by a Balkan expansion[22]. The rapid colonization of northern Europe by alder from a north Balkan source seen in the pollen fits well with the genetic evidence, but the chloroplast DNA haplotypes show several more distinct southern refugia indiscernible in pollen[28].

Erinaceus europeus, the western European hedgehog is parapatric with its sibling species *Erinaceus concolor* in the east. After an intriguing allozyme study, its phylogeography has been examined with sequences from the cytochrome (cyt) b mtDNA gene[29]. Parsimony and distance trees both show considerable divergence between the two species, and surprisingly reveal distinct clades within these. The depth of divergence between these lineages suggests separations several million years ago, possibly at the beginning of the Quaternary. The hedgehog genome across Europe is thus divided into three principal north/south strips, with a fourth clade in Turkey and Israel. This pattern is quite different from the grasshopper and points to the colonization of northern Europe from three glacial refugia, a western/Spanish one, a central/Italian one and an eastern/Balkan one (Fig. 2). Turkey was also a refugium, and more data may show further subdivisions, particularly in the south and east. Colonization patterns similar to the hedgehog are seen in the oaks and silver fir, with genomes from several southern refugia contributing to the postglacial advance to the north. The populations of oaks emerging from their last glacial refugia probably contained different mixtures of more ancient chloroplast DNAs[22]. A narrow hybrid zone in Finland between western and eastern cytotypes indicates colonization by very different routes[30].

Ursus arctos, the brown bear, has had its broad European distribution reduced by man to a few isolates in the west, with larger populations in Scandinavia, eastern Europe and Russia. Consequently it is the object of conservation efforts. Analysis of the mtDNA control region revealed distinct eastern and western lineages[31] and *U. arctos* appears to have colonized most of Europe from an Iberian and a Caucasian/Carpathian refuge[22] (Fig 2). These expansions met in central Sweden, probably as the last of the Scandinavian ice cap melted ~9 kyr ago, and formed a hybrid zone. The western mtDNA lineage is subdivided, but the clades from Italy and the Balkans did not expand into northern Europe, possibly prevented by the prior occupation of a rapid expansion from the other refugia. From present genetic data, it is not clear exactly from where the eastern expansion emanated, nor how far west it spread across Europe. This latter issue may be addressed through studies of ancient DNA. The bear pattern with its east/west embrace of Europe is similar to that found in shrews and water voles[22,25]. The presence of several small mammal hybrid zones in the same place in Sweden as that of the bear[32] clearly indicates distinct eastern and western colonizations as the ice melted. All three species had distinct genomes that remained in Italy and probably other parts of southern Europe. Studies on other species are in progress, and it will be informative to compare them with these broad paradigm patterns. We can expect individual variants, and maybe a strikingly different one exists.

Table 1 The rates of evolution of various DNA markers

DNA marker	Organism	Average rate of divergence*
Nuclear DNA		
Synonymous (silent) sites	Mammals	0.7
	Drosophila	3.12
	Plant (monocot)	0.114
Intron	Mammals	0.7
Chloroplast DNA		
Synonymous (silent) sites	Plant (Angiosperm)	0.024–0.116
Mitochondrial DNA		
Protein-coding regions	Mammals	2.0
	Drosophila	2.0
COI	Shrimps	1.4
D-loop	Human	14
	Human	17.5
	Human	270
Synonymous (silent) sites	Plant (Angiosperm)	0.01–0.042
Microsatellite		
	Human	5.6×10^{-4}
	Drosophila	6.3×10^{-4}

* Rates of evolution of various DNA markers are given as per cent divergence per million years, which for microsatellites is in units of mutation per locus per gamete per generation (for details, see refs 84–86).

Europe—emerging features

It appears that the Balkans were a source for all species in the east and for many species in the west; the grasshopper/alder/beach/newt pattern would seem to be common; conversely, Italian genomes rarely populated northern Europe, for example, hedgehog and oak. It seems that the ice-capped Alps were an initial barrier to their northward expansion, while the Pyrenees apparently acted as a barrier in fewer cases, for example, grasshopper, alder and beech. No firm generalizations can yet be made about colonization pattern from species attributes, such as dispersal powers, generation time or habitat adaptation. More species need to be examined.

When these expanding genomes met they formed hybrid zones, and many of these have been described across Europe[12]. These appear to cluster in the Alps and central Europe, and to some extent in the north Balkans and Pyrenees[13,15,22,25]. Remington[33] called such clusters "suture zones" from his review of North American species. The neat cluster of zones in central Sweden was probably produced by the final melting of the Scandinavian ice cap, which allowed eastern and western colonists to meet (Fig. 3). For the Balkans, more genetic data is needed; they appear genomically diverse and dissected, and the source of most northward postglacial expansions.

Britain received oaks, shrews, hedgehogs and bears from Spain, but grasshoppers, alder, beech and newts from the Balkans. The

mixture in Sweden is even more complex. This causes one to pause in considering coevolutionary relationships and community structure, both in terms of their evolution and stability. Furthermore, there is fossil evidence for very different communities in refugia and during the many range changes.

Partly because of these glacially induced range changes, northern Europe has less genetic variety than southern Europe in terms of numbers of species, subspecific divisions and allelic diversity: "southern richness to northern purity," so to speak[15]. Rapid expansion may well explain the loss of alleles in northern populations, whereas the varied topography of southern parts provided suitable habitats through several glacial cycles, thereby allowing the divergence and accumulation of several genomes[15,22]. This process may continue to produce species ultimately. It has significant conservation implications.

Some species have managed to maintain themselves in southern Europe for many ice ages, whereas others have colonized or recolonized more recently (Table 2). The toad *Bombina* and hedgehog may well have been diverging for 5 Myr, but the eastern and western genomes of the grasshopper and the bear probably first entered their refugia about 0.4 Myr—four ice ages ago—a time of Red Sea salinity[10] and a long cold period. It may be that a number of species became extinct in Europe, even in their southern peninsular refugia. They recolonized these after this and have been expanding, contracting and diverging since. Genomes surviving in these refugial areas thus diverge by repeated allopatry, protected by hybrid zones[12,13], and may speciate in one or over many ice ages.

Other parts of the world

The type of consideration using molecular phylogeographies for Europe can be applied to other parts of the world, bearing in mind the differences in geography and climatic history, to seek possible generalities. In such a review only some points can be highlighted.

North America. Although a continent of similar size and at similar latitude, North America differs significantly from Europe in geographical features that should have affected the structuring of genomes and species by the climatic oscillations of the Quaternary. Altitudinal shifts, north/south range changes and recolonization from outside would be different. The ice sheets advanced further south to 40 °N, and the tundra belt was much narrower in the east. The mountainous west was more varied with ice, tundra, pluvial lakes and deserts, and the Bering land bridge connected Alaska and Siberia[2].

There is an increasing flow of phylogeographic papers using molecular markers in North American species[34], and only a few cases can be mentioned here. The seminal comparative studies were on marine and coastal species in southeastern USA[35], where northern and southern DNA lineages made contact around Florida. Interestingly, this well studied region contains one of the "suture zones between recently joined biotas" that Remington[33] proposed for terrestrial organisms, and parapatric mtDNA clades have been described for several land species here[36]. These terrestrial zones may be analogous to those in southern Europe where the northern expanding taxa blocked the advance of those from the south. A similar explanation is possible for the suture zones that run from New England to the Dakotas, which broadly coincide with the edge of the ice sheets. The southeastern states also show a suture zone for freshwater fishes, where western and eastern drainages and genomes meet around the Alabama–Georgia line in the southern Appalachians[36]. The turtles of this region provide a fine example of how phylogeographic concordance can demonstrate shared biogeographical histories[37,38]. The considerable species richness and substructure in southeastern USA was probably generated by the survival and divergence of genomes in refugia, as ranges changed through repeated glacials and interglacials, with changes in sea level, ocean currents, peninsular climate and water drainages. Such southern richness is found in Europe, but the Appalachians run north/south and possibly played a different role from the European mountains.

Many North American studies report lower genetic diversity in northern populations that have expanded from refugia south of the ice sheets[15,39,40], and also greater clade area in fish[16]. As the ice melted it produced large flooding proglacial lakes, which could have dispersed aquatic organisms extensively in both Nearctic and Palaearctic. Plant and animal species in the Pacific northwest show the southern richness and northern purity produced by the retreat of the ice sheets[39,40], and greatly strengthen the conclusions drawn from the northern colonization of Europe[14,15]. Interestingly, several widespread North American birds have low genetic diversity across their range reflecting a rapid postglacial expansion, but several other species show genome subdivision in the west and southwest USA[41]. Indeed, these parts are now and were during glaciations dissected by mountains and deserts, which produced complex habitat shifts, long-term isolation and the genome divergence that is seen in a number of species[42].

The Bering land bridge was free of ice during the glaciation, and genetic phylogenies show that Berigian refugial genomes colonized deglaciated regions of North America in some fish and beetles[16,43]. Phylogeographic information is becoming available for a range of other Arctic organisms, some circumpolar, which shows that their genomes have been fragmented by glaciations to produce subspecies and in some cases species. These include guillemots[44], dunlins[45], reindeer[46] and bears[47,48].

It would seem useful to see North America as principal regions differing in geography, climate and hence species history and genomic pattern. The deglaciated northern parts with a distinctive northwest region most resemble Europe, whereas the well-studied southeast would seem richer than southern Europe because of the

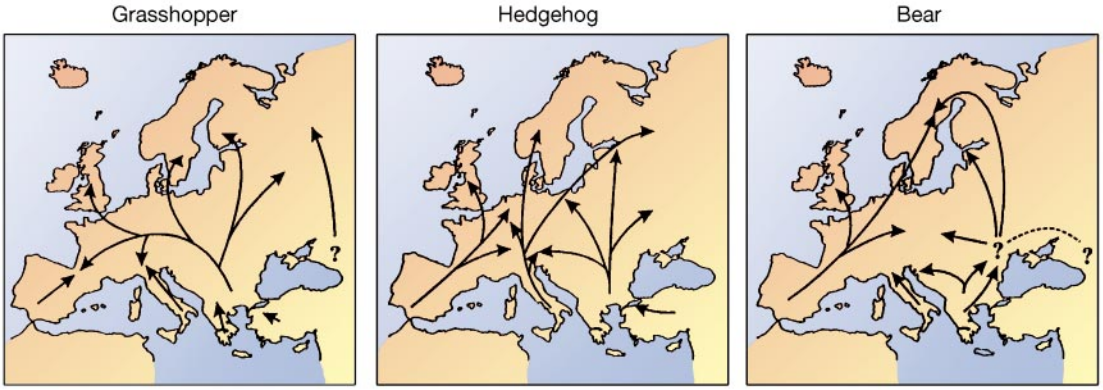

Figure 2 Three paradigm postglacial colonizations from southern Europe deduced from DNA differences for the grasshopper, *Chorthippus parallelus*, the hedgehog, *Erinaceus europeus/concolor*, and the bear, *Ursos arctos*. The main refugial areas, Iberia, Italy, the Balkans and Caucasus, contributed differently to the repopulation of northern parts.

presence of the Mediterranean and North Africa at similar latitudes. The complex southwest has no real European analogue, except perhaps Iberia. More full species phylogeographies are needed to build regional syntheses and indicate their general principles.

Tropical latitudes. Conditions during the last ice age were colder and drier in the tropics, extending deserts and savannah while reducing rain forests. Unfortunately, these have a poor pollen record, but some recent studies in Amazonia and Queensland suggest that the rain forest species survived in many local wet places and along moist gullies[2,49]. Apparently, temperatures were 6 °C lower in the mountains, and forest species descended to the lowlands. The tropics are particularly speciose, and so the combined investigation of palaeoclimatology, palaeontology and molecular phylogeography, as in Europe and North America, promises to be interesting and important.

There are already a few revealing studies in Australia, Amazonia, Africa and southeast Asia. Climatic oscillations have expanded and dissected the strip of rain forest in northeast Queensland and with it the species there. Several birds, reptiles and frogs show low mtDNA diversity as the result of population contractions, whereas some have retained overall diversity through different haplotypes surviving in several patches. Their phylogeographies reveal a concordant divide between north and south clusters, which fits with an initial separation at the start of the Quaternary[50]. Amazonia poses a particularly difficult task owing to its large size and inadequate palynology, taxonomy and sampling[51]. So the comparative phylogeography of 35 species of small mammals for 15 genera using mtDNA is most welcome[52]. Surprisingly, many taxa have deep sequence divergence dating to the Pliocene. The Pleistocene Andes uplift producing the Iquitos Arch across the Jura river is coincident with phylogenetic breaks in 11 of the taxa, although genome divergence at the major rivers themselves does not support the river barrier hypothesis for Amazonian speciation.

There are studies emerging for a range of organisms in Central America and the Caribbean, particularly in birds and fish. For freshwater fishes, the picture looks complex with several overlaid cycles of colonization and lineage extinction from the late Miocene through the Pleistocene[53].

Savannah. While the tropical forests were reduced during the Quaternary ice ages, the drier climate allowed grassland and savannah to expand, so that species associated with these habitats had a different cycle of contraction into refugia. Several African bovids inhabit the Savannah, and recent mtDNA phylogeographies of the hartebeest, topi and wildebeest show how the climatic oscillations affected their distribution and genomic divergence[54]. The first fossils of these species are reported from the early Quaternary, and distinct monophyletic mtDNA clades indicate that their Africa-wide distribution has been reduced several times to a few refugia. The asymmetric gene tree topology of the wildebeest and a decline in sequence diversity from South Africa to east Africa clearly suggest that it colonized from South Africa in recent cold dry periods. The first fossils are from east Africa, but this population probably did not survive and the region was recolonized. Clearly, the careful combination of climatic, fossil and genetic evidence can provide a much clearer picture of the evolution in these habitats also.

Tropical mountains. Like the small mammals in Amazonia, bird species in lowland tropical forest of South America and Africa are quite old with more than 6 Myr of DNA divergence[55,56]. In contrast, mountain regions in the tropics contain clusters of recently diverged lineages along with older species. So it has been proposed that these mountains provide through the ice ages a stable moist habitat, in which older species may survive and new lineages be generated. More evidence for tropical mountains as centres for bird speciation through the Pleistocene climatic changes comes from mtDNA divergence in greenbuls from east Africa[57] and spinetails from the Andes[58]. As suggested for southern Europe, these refugial and speciation properties of the mountains may derive from their topographic variety, which allows habitats and lineages to persist by altitudinal shifts and also diverge because of distributional dissection.

Wallacea. The lowering of the sea level during the ice ages produced a number of land bridges and sea barriers. The Sunda shelf in southeast Asia is perhaps the largest of these, and linked much of Malaysia and Indonesia west of the Wallace line to the Asian mainland. The straits of Malacca between Malaya and Sumatra are both narrow and shallow, so even small falls in sea level would produce a land bridge. Consequently, this region will have been dissected and rejoined on many occasions by climatic oscillations back into the Pliocene affecting the distribution and evolution of species.

Some mtDNA investigations show deep divergences that would suggest Pliocene and early Pleistocene radiations. In the case of the spineless hedgehog, *Hylomys*, there are distinct mainland and island clades[59], whereas the river catfish ancestral lineages form three regional groups[60]. The mtDNA of the striped rabbit, *Nesolagus*, in Sumatra is very different from that of newly discovered representatives in Laos[61]. These data suggest there has been no admixture of

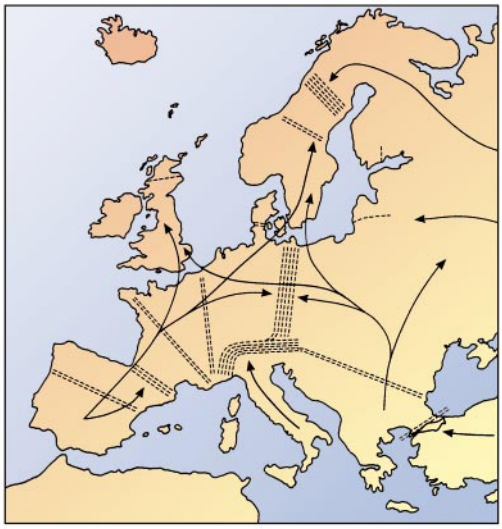

Figure 3 The general position of some well-known hybrid zones in Europe, which show major clustering in Scandinavia, central Europe and the Alps. Other clusters are apparent in the Pyrenees and the Balkans. These suture zones are caused by commonalities of ice-age refugia, rate of postglacial expansion and physical barriers. There is further subdivision in the southern regions.

Table 2 DNA sequence divergence and maximum time of separation in species groups that colonized Europe after the last ice age

Organism	DNA sequence	Divergence	Maximum age	Refugia*
Bombina bombina 'Fire bellied toad'	mt RFLP	9.4%	5 Myr	(I) B B
Erinaceus europaeus 'Hedgehog'	mt cyt b	6–12%	3–6 Myr	S I B
Triturus cristatus 'Crested newts'	mt RFLP	4–8%	2–4 Myr	S I B
Arvicola terrestris 'Water vole'	mt cyt b	4–7.6%	2–4 Myr	S I B
Crocidura suaveolens 'White toothed shrew'	mt cyt b	3–6.4%	1.5–3.2 Myr	S (I) B
Mus musculus 'House mouse'	mt RFLP	3.4%	1.7 Myr	W & E
Microtus agrestis 'Field vole'	mt RFLP	2%	1 Myr	(S) B E
Sorex araneus 'Red toothed shrew'	mt cyt b	1–3.8%	0.5–2 Myr	S (I) B
Ursus arctos 'Brown bear'	mt control region	2.7–7%	0.35–0.85 Myr	S (I) B
Chorthippus parallelus 'Meadow gresshopper'	mt 6.7 kb	0.7–0.9%	0.3–0.5 Myr	(S) (I) B

*Deduced southern refugia of distinct genomes are given. S, Iberia; I, Italy; B. Balkans; E, east; W, west. Brackets show ones that did not expand out (see ref. 22 for details). RFLP, restriction-fragment length polymorphism.

populations during the many Pleistocene land-joining. Other diverged species have been discovered from the mountains of this region.

A different pattern is seen in the mtDNA of the flying fruit bat, *Eonyeteris*, along the Indonesian archipelago. Sequence divergence suggests events in the Pleistocene and also reflects the sea crossing distance between islands, with a decreasing diversity from ancestral western populations to colonized eastern ones. There are indications that this may be another emerging concordant pattern in the fauna of these islands[62].

Seas and oceans. For several marine species the Indo/west Pacific region, which includes the Sunda Shelf and Indonesia to the Torres Straits, marks a phylogenetic disjunction between Indian and Pacific lineages and taxa. This includes butterflyfish, starfish, damselfish and coconut crab, and coastal mangroves and fishes[63–66]. The depth of mtDNA and allozyme divergence would place the effective separation of these sister taxa in the Pleistocene, which is when the sea level was lowered during ice ages and the Asian–Australian archipelagos were more of a barrier. As with terrestrial and freshwater organisms, the Indo/west Pacific seas are rich in species, which may be due to the many changes in distribution caused by the sea level oscillations in the topographically complex archipelagoes. It may function as a species source and refuge in a similar way to mountainous regions in lower latitudes.

From studies so far it seems that species in the large oceans show little geographic genetic differentiation, as exemplified in the butterfly fish in the tropical Pacific[63]. In temperate waters, coastal sardine taxa from separate parts of north and south Pacific and Indian Oceans from mtDNA sequences would appear to have diverged within the past 0.5 Myr, with each geographic form carrying the genetic signature of rapid expansion from a founding colony[67]. A survey has revealed that pelagic fishes in general, with their high dispersal and large but fluctuating population sizes, show low genetic differentiation among geographic regions; the 21 species ranged from tuna to anchovies[68]. Such low genetic diversity might be expected for temperate species that have undergone range changes due to the ice ages, but it is not yet clear how this compares with their refugial areas at lower latitudes and in the Tropics.

Quaternary speciation

Increasing climatic oscillations during the past 3 Myr have driven many range changes in all parts of the world. It was necessary to move, adapt or go extinct, and present lineages had the ability and luck to survive such shifts. Each time they would colonize new territory, face new environments and meet new neighbours. These challenges would cause genomes to diverge, both through selection and chance, and ultimately speciate[13]. The time taken to speciation and hence the rate of speciation are currently of some interest.

It was generally thought that the Pleistocene increased the initiation of species, but a compilation of molecular data for divergence in subspecies and species complexes showed that species were formed through the Pliocene and Pleistocene[15]. A more recent analysis of available molecular differences among lineages and sister species of some 431 birds, mammals, frogs, reptiles and fish shows that genetic divergence between sister species ranges fairly evenly through the Pleistocene and Pliocene[69]. Such analysis has been applied specifically to North American birds[70]. On average, but with considerable variation and depending on species definition, speciation duration is about 1–2 Myr, and speciation appears to have been proceeding unhindered through the Quaternary period.

There is now clear evidence of extremely rapid differentiation in insects, lizards and fish in new habitats[71]. From molecular data the sympatric ecomorph species of sticklebacks, arctic charr and lake whitefish were probably formed in lakes as the ice retreated[16,72]. On the other hand sister taxa of some species for which molecular data shows millions of years of divergence still hybridize, for example, toads and newts.

The current evidence indicates that although genomic divergence proceeds over millions of years, morphological, physiological and behavioural changes may produce subspecies and species at almost any time.

Many of these new local species will become extinct with the next major climatic shift, when their present location is covered by ice, tundra, savannah or desert, for example. By good fortune one may move and survive to found a more extensive new taxon. In analysing DNA divergences over the Plio-Pleistocene, we are observing lineages that have survived many ice ages. It is a species survival rate—a product of high speciation rate and periodic extinction. Mountain regions in the tropics have a greater range of life-supporting environments with greater continuity through climatic shifts, which would provide more opportunity for newly formed species to survive. This may partly explain greater species richness in the tropics.

Modern man and the ice ages

The ice ages can be expected to have also affected the evolution of man. Molecular genetic and archaeological studies are now being joined to elucidate the origin and spread of modern man worldwide. Most evidence supports an African origin followed by an expansion to other parts[23,73–76]. The bones and tools of forms of *Homo erectus* are found in Africa and over Eurasia from 1.5 Myr ago. In Europe these produced Neanderthals from 300 kyr ago. DNA sequence from a fossil bone places their divergence about 600 kyr ago, with no evidence of similar sequences in present human data[77]. Archaic *Homo sapiens* fossils and tools occur in Africa from 500 kyr ago, progressing to those of modern *H. sapiens* from 200 kyr to 100 kyr ago; a period coincident with the major ice ages.

A number of recent studies on molecular genetic diversity in present populations argue for an effective population size of about 10,000 in Africa during the Upper Pleistocene, through what is called a long bottleneck[23]. These include mtDNA, Y chromosome, Alu insertion, human lymphocyte antigen (HLA), β-globin, the zinc-finger gene on the X chromosome (ZFX) intron and microsatellite coalescent estimates. Mismatch distributions and neighbour-joining dendrograms suggest a population expansion ~100–50 kyr ago in Africa and 23 kyr ago in Europe[73]. From ~130 kyr to 71 kyr (OIS5) ago, during and following the last interglacial, the climate was fairly warm with some reversals, and modern man entered the Levant[78]. Around 71 kyr ago, the GRIP ice core record shows a very cold spell that is coincident with the volcanic eruption of Mount Toba in Sumatra, the largest anywhere for 450 Myr. It is argued by Ambrose[79] that this reduced the population of modern man to tropical refugia from which he expanded ~55 kyr ago. Significantly, Neanderthals who were probably better adapted for cold climates moved into the Levant from 71 kyr ago when modern man vacated it[78].

Modern man entered Europe from the Levant about 41 kyr ago with new technology and social structure, spreading across southern parts and replacing the Neanderthals gradually[80]. The Neolithic agricultural revolution spread from the Near East around 9–5 kyr ago in the warm climate after the Younger Dryas cold reversal. This advance is correlated with clines in classical gene frequencies[81]. Artefacts show range changes between his arrival and this later spread, and man would have been pushed to southern Europe at the height of the ice age 24–20 kyr ago Mitochondrial DNA data of modern populations[82,83] show six principal lineages of this Upper Palaeolithic age, which have different divergences. The question being addressed now is how much of our genome originates from these earlier ice-age settlers: the contribution from the Neolithic revolution may be less than we thought. □

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