Genetic consequences of climatic oscillations in the Quaternary

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An appreciation of the scale and frequency of climatic oscillations in the past few million years is modifying our views on how evolution proceeds. Such major events caused extinction and repeated changes in the ranges of those taxa that survived. Their spatial effects depend on latitude and topography, with extensive extinction and recolonization in higher latitudes and altitudinal shifts and complex refugia nearer the tropics. The associated population dynamics varied with life history and geography, and the present genetic constitution of the populations and species carry attenuated signals of these past dynamics. Phylogeographic studies with DNA have burgeoned recently and studies are reviewed from the arctic, temperate and tropical regions, seeking commonalities of cause in the resulting genetic patterns.

Arctic species show distinct shallow genetic clades with common geographical boundaries. Thus Beringia is distinct phylogeographically, but its role as a refugial source is complex. Arctic taxa do not show the common genetic pattern of southern richness and northern purity in north-temperate species. Temperate refugial regions in Europe and North America show relatively deep DNA divergence for many taxa, indicating their presence over several Ice Ages, and suggesting a mode of speciation by repeated allopatry. DNA evidence indicates temperate species in Europe had different patterns of postglacial colonization across the same area and different ones in previous oscillations, whereas the northwest region of North America was colonized from the north, east and south. Tropical montane regions contain deeply diverged lineages, often in a relatively small geographical area, suggesting their survival there from the Pliocene. Our poor understanding of refugial biodiversity would benefit from further combined fossil and genetic studies.

Keywords: phylogeography; Ice Ages; refugia; colonization; hybrid zones; biodiversity

1. INTRODUCTION

Our climate has been cooling for *ca.* 60 Myr, with the Antarctic ice sheet forming *ca.* 35 Ma and the Arctic icecap growing from *ca.* 3 Ma. The Quaternary Period has been dominated by Ice Ages, which involve repeated global cooling and increasing advances of these ice sheets. These oscillations are paced by regular eccentricities in the Earth's orbit around the sun every 100, 41 and 21 kyr. The large ice sheets, surrounding permafrost, lower global temperature and reduced water availability caused great changes in the distribution of species, which can be seen in the fossil record (Bennett 1997; Williams *et al.* 1998). Recent work with cores from ice sheets and sea beds confirms the effects of millennial-scale change in climate nested within the main 100 kyr cycle. These involved changes of as much as 7–15 °C over a few decades, which then lasted for hundreds of years, and there is fossil evidence that these, including the Younger Dryas *ca.* 11 ka, caused shifts in species distributions.

The effects of Ice Ages on species ranges varied with latitude and topography. High latitudes were covered with ice or with permafrost, and temperate and tropical regions were compressed towards the equator. Increased aridity reduced tropical forests considerably, and deserts expanded. Mountain regions between the fortieth parallels were significant because, given available lower land, montane species could descend as the climate cooled and survive the oscillations in the same region. Also, mountains like the Alps and Appalachians, and waters like the Mediterranean and Gulf of Mexico, could serve as barriers to species movement. The lowered sea-level uncovered *ca.* 20% more land, which in places like the Bering Strait, the Sunda Shelf and the China Sea acted as bridges. The extent of the range changes depended on individual species migration and adaptations, but they were major and repeated (Hewitt 2003).

Such latitudinal and altitudinal range shifts involved considerable demographic changes and provided opportunities for adaptation to occur. These will clearly have stochastic and selective effects on genetic variation and architecture. Populations and lineages will go extinct, alleles will be lost in bottlenecks and founder events, mutations will occur through time and be spread by selection and population expansions. The past 15 years have seen great advances in how these events can be studied, and in understanding how their genetic consequences can be used to interpret evolutionary history. Primary among these is the ability to obtain DNA sequences readily from most species across their geographical range. Animal mtDNA, cpDNA and non-coding nuclear regions are most favoured for Quaternary studies providing suitably variable sequences. Other hypervariable markers such as microsatellites and AFLPs are usefully employed for

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studies of recent events, but they suffer from homoplasy and do not provide clear genealogies. (For a brief introduction and primary references to the various molecular markers employed, their rates of evolution and their analysis, see Hewitt (2001) and Hewitt & Ibrahim (2001).)

The DNA haplotype sequences obtained may be joined by mutational steps into a network, and a gene phylogeny produced in combination with closely related taxa. When this gene tree is placed in a geographical context it has great explanatory potential, creating the field of phylogeography (Avise *et al.* 1987; Avise 2000). The availability of such information-rich DNA sequences has encouraged the application of existing population genetic and spatial methods of analysis, and the development of new ones. This is a very active field, which includes for example, genetic demography (Harpending *et al.* 1998; Emerson *et al.* 2001), statistical phylogeography (Knowles & Maddison 2002) and landscape genetics (Manel *et al.* 2003). Combination of such techniques is being sought to extract the data's full information, and so test geographical evolutionary models.

Studies of phylogeography have grown exponentially since its naming (Avise *et al.* 1987; Avise 1998; Stone 2000), particularly in North America and Europe, from which some basic patterns have emerged and concepts have been developed (Avise 2000; Hewitt 2000). Because this field has grown beyond the point where the biogeography of the whole world can be fully discussed here, the main focus of this review is on northern latitudes, where there have been so many recent papers. The tropics are far more diverse and some reviews are mentioned. An attempt will be made to outline the main processes and conclusions so far, and highlight some currently interesting areas where the Quaternary Ice Ages have had genetic impact.

2. TEMPERATE EUROPE

Quaternary phylogeography in Europe and North America is based on fossil data, particularly the extensive network of pollen cores, which describe the changes in distribution of species through the recent Ice Ages (Huntley & Birks 1983; Bennett 1997; Hewitt 1999). This is most detailed for the last cycle (120 kyr), where it is complemented by recent ice-core data on palaeoclimates. A few cores go back several Ice Ages, but earlier palaeobiological reconstructions of distributions are necessarily less certain. At the LGM (23–18 ka) the European ice sheet extended south to 52° N and permafrost south to 47° N. As the ice melted, species from the tundra, cool temperate and warm temperate habitats moved north. For most temperate species their LGM refugia were in the south of Europe and the colonization northwards was rapid (Bennett 1997; Hewitt 1999).

By using DNA haplotype similarity it is possible to deduce which northern populations of a species are recently related to more southerly ones that now inhabit areas near LGM refugia. The southern peninsulas of Iberia, Italy and the Balkans–Greece, along with the Caspian/Caucasus region acted as refugia, and many species show taxonomic and genetic diversity in and among these regions. The colonization patterns of northern parts by these distinct genomes can be determined by using

suitably variable sequences with wide range sampling (Hewitt 1996, 1999; Taberlet *et al.* 1998). The number of such studies is not great, but has grown significantly in the past 2 years. In principle, a species could colonize Europe from any of these four major temperate refugia; in fact, DNA relationships show that in most species Balkan genomes predominated, then Iberian, and then Italian, with the contribution from the east being undetermined in some species. This produced subdivisions of species into a patchwork of genomes, often delimited with sharp hybrid zones, and this adds much finer resolution to the fossil record of these events. In Europe these hybrid zones are often clustered into suture zones along the Alps and Pyrenees, down central Europe and across Scandinavia, where many expanding genomes met. Such suture zones are also a feature in other parts of the world (Hewitt 2000).

These general postglacial colonization patterns from southern refugia by land were exemplified by those of the grasshopper *Chorthippus parallelus*, the hedgehog *Erinaceus europaeus/concolor*, and the bear *Ursus arctos*, with more or less similar ones in other species (figure 1) (Hewitt 1999). Since then, many similar and variant species' phylogeographies have been added. This is particularly so for freshwater fishes, where Europe's rivers have often been colonized from the Black Sea up rivers like the Danube and the Dneiper. Perhaps they deserve a new paradigm, illustrated by the perch or chub (table 1; figure 1). These fishes contain several lineages with divergences indicating disjunctions over the past 2 Myr. Many of these have clear geographical distributions and provide evidence of older Black Sea–Caspian Sea divergence. They also indicate colonization of western Europe in recent interglacials and postglacial expansions from several major refugia, particularly the Black Sea, Dneiper–Volga, Danube, Rhine–Rhone, Elbe and other rivers. This varies in detail among species, with those more cold-adapted, like the bullhead, having more northerly glacial refugia. The influence of older water bodies like the Ponto–Caspian Sea and recent great periglacial lakes and floods is also apparent. Further east, the application of DNA phylogeography with discerning analysis to the grayling (*Thymallus* spp.) of Lake Baikal is providing new evidence and novel insights on these ancient waters (Koskinen *et al.* 2002). Whereas the major rivers contain old Pliocene lineages, Lake Baikal's current lineage colonized recently, *ca.* 110–450 ka during recent Ice Age cycles.

Modelling and simulation of leptokurtic expansion from the most northerly LGM populations produces reduced allele diversity and areas of genetic homogeneity (Ibrahim *et al.* 1996), and this genetic pattern has been reported in many species from Europe and North America (see, for example, Hewitt 1996; Soltis *et al.* 1997; Bernatchez & Wilson 1998; Schmitt *et al.* 2002; Michaux *et al.* 2003). Such leading-edge colonization has the corollary that it is more difficult for southerly populations to expand once the space has been filled (Hewitt 1993), and the greater number and smaller range of southern genomes support this.

Not all temperate species followed a rapid form of expansion, as is clear from the fossil record. Some were dependent on particular conditions or on other species, and some were more affected by various barriers, habitat
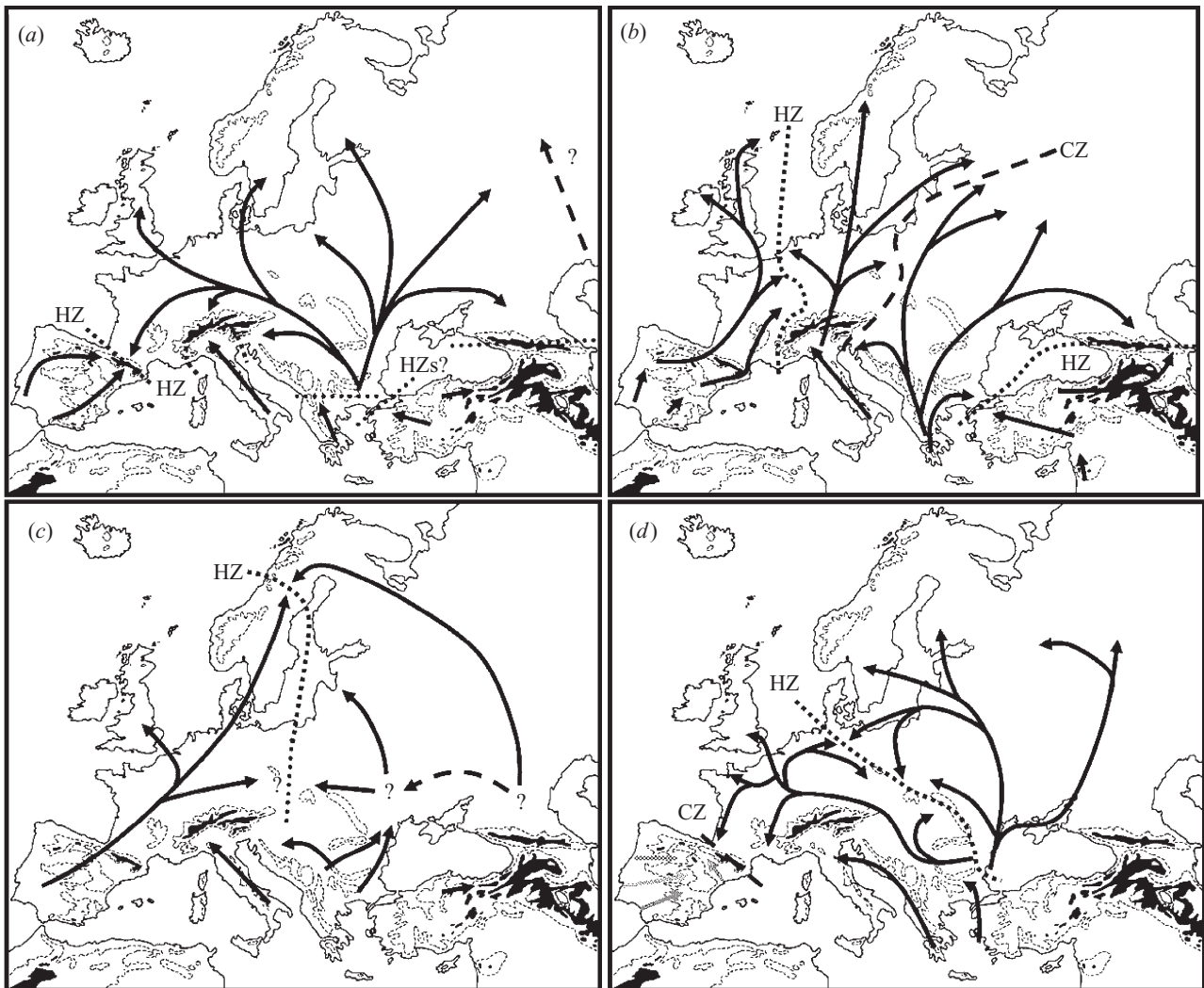
Figure 1. Putative postglacial colonization pathways from refugial areas for four paradigm species: (*a*) *Chorthippus parallelus*; (*b*) *Erinaceus europaeus/concolor*; (*c*) *Ursus arctos*; and (*d*) *Leuciscus cephalus*, as deduced from DNA haplotype relationships. Contact zones (CZ) between nominal species are indicated by dashes and hybrid zones (HZ) between genomes are given as dotted lines.

distributions and prior colonizers. Such slower expansions would involve shorter dispersal and larger effective population sizes, which would tend to retain genetic diversity, and would be expected in the mountain blocks of southern temperate regions and the tropics (Hewitt 1996). These two extreme modes of colonization, termed Pioneer and Phalanx (Nichols & Hewitt 1994), when combined in different habitat distributions and climatic oscillations can produce a variety of geographical genetic structures. Long-distance dispersal also produced small pockets with distinct genomes well ahead of the main population, which could then expand to give a patchwork. This aspect has been significantly extended to examine the causes of the local distribution of oak (*Quercus* spp.) genomes that colonized Europe from the south (Petit *et al.* 2001). In deciphering the phylogeographical signals of a species it is necessary to consider its natural history and how it expanded and contracted in its particular range.

A feature of temperate species in southern Europe is the presence of several distinct geographical genomes with a variety of alleles. This richness is also seen at the subspecific and specific taxonomic levels, and it probably accumulated over several Ice Ages (Hewitt 1999). The toad

Bombina and hedgehog *Erinaceus* contain genomes that would have diverged in the Pliocene, whereas the grasshopper *C. parallelus* and the bear *U. arctos* show shallower divergence of a few Ice Ages. The association of these distinct genomes with major peninsulas argues that these have been glacial refugia for these species lineages repeatedly. Hedgehogs from Sicily have a divergent mtDNA haplotype, which indicates its survival from earlier in the Quaternary; the rest of Italy produced younger clades that colonized northwards in more recent interglacials (Santucci *et al.* 1998; Seddon *et al.* 2001). A distinct Sicilian lineage also occurs in the woodmouse *Apodemus sylvaticus* (Michaux *et al.* 2003). Such remnant populations from previous Ice Ages are also seen in the pond turtle *Emys orbicularis* (Lenk *et al.* 1999). In the salamander *Salamandra salamandra* (Steinfartz *et al.* 2000), pockets of older orphan lineages are found in Spain and Italy surrounded by more recent expansions that populate the north. Despite nice clues like these, the phylogenetic history of such regions is not as easy to unravel as those farther north that were covered by postglacial expansions. Recent studies of a range of organisms in southern regions are instructive, particularly in Iberia, which was an

Table 1. Freshwater fish species with broad European ranges showing distinct phylogeographical patterns, with some indication of their putative divergence times derived from DNA divergence, glacial refugia and postglacial colonization routes. cytb, D-loop and CR are sections of the mtDNA genome. See references for details.

species	range	marker	phylogenetic structure	divergence time (Ma)	refugia	colonization	references
Leuciscus cephalus, chub	Sweden–Urals Turkey	mtDNA cytb	four lineages 5.2–7.9%	0.5–3.5	west and east Balkans Danube, Black/Caspian	Danube west Dneiper-Volga north and west	Durand *et al.* (1999)
Perca fluviatilis, perch	Norway–Siberia Mediterranean	mtDNA D-loop	four lineages, 0.7–1.3%	0.2–0.6	Dneiper Danube/Rhine Black Sea	Dneiper northwest Rhine north and south Black Sea west	Nesbo *et al.* (1999)
Barbus barbus, barbel	England–Black Sea Middle Europe	mtDNA cytb	two lineages, 0.2–1.5%	0.1–0.8	Rhine/Rhone Danube Black Sea	Rhine south Danube west	Kotlik & Berrebi (2001)
Salmo trutta, brown trout	Iceland–Afghanistan Atlas	mtDNA, RFLP	five lineages, 1.2–2.2%	0.5–2.0	Atlantic, Mediterranean, Black/Caspian	Atantic north and northeast Adriatic west and east Black Sea west	Bernatchez (2001)
Cottus gobio, bullhead	northwest Palaearctic North Europe south Alps	mtDNA, CR allozymes	seven lineages 0.6–3.9%	0.1–0.5	Adour, Loire Seine, Rhine Elbe, Danube Dneiper	Rhine west and south Elbe north Danube west and north Dneiper north	Volckaert *et al.* (2002); Hanfling *et al.* (2002)

important refugial area (Gomez & Lunt 2004). These include rotifers (Gomez & Lunt 2004), leaf beetles (Gomez-Zurita *et al.* 2000), lizards (Paulo *et al.* 2002), salamanders (Alexandrino *et al.* 2002) and plants (Gutierrez Larena *et al.* 2002). They reveal the survival of deep Pliocene lineages, often in mountain blocks, indicating altitudinal shifts with expansions and contractions through the Ice Ages.

For many species the postglacial expansion has apparently occurred from a few populations on the northern edge of their refugial LGM distribution. Just where these founding populations were and how they expanded depends on the adaptations of the individual species, the distribution of its habitat and how that changed with climate. Species with more temperate adaptations would have refugial ranges further south in general than those more cold-hardy. For example in small mammals, species with southern and northern temperate species ranges might be the woodmouse (Michaux *et al.* 2003) and the field vole, *Microtus agrestis* (Jaarola & Searle 2002). Clearly a good fossil record is important in determining the limits of refugial ranges (Tzedakis *et al.* 2002).

The survival of populations in these southern refugial peninsulas over few to many Ice Ages, while peripheral areas are colonized and cleared repeatedly, allowed their lineages to diverge and accumulate genetic differences, and they may ultimately become species. It is a form of repeated allopatry, and speciation may be accelerated by selection for different adaptations (Hewitt 1996, 1999). There is evidence that speciation has been accomplished postglacially under strong divergent selection in certain cases, whereas other taxa retain the ability to hybridize despite divergence since the Pliocene. Compilations of molecular differences in races, subspecies and species complexes indicate that speciation has been occurring through the Pliocene and Quaternary at various levels of genetic divergence (Avise *et al.* 1998; Klicka & Zink 1999; Hewitt 2000).

3. THE ALPS

Mountain ranges are seen as important for divergence and speciation, both in the tropics (Fjeldsa & Lovett 1997) and in more temperate regions (Hewitt 1996, 2000; Knowles 2000), and the Alps are a major feature in moulding the phylogeography of European species. Their present climate ranges from mediterranean to glacial, they are topographically diverse, and they have acted as a barrier and a refuge through climatically induced range changes. Many present-day species in the region colonized their present ranges from lower altitudes and latitudes, whereas those with Arctic–Alpine distributions that were not annihilated descended during the Ice Ages and may have spread more widely across the cold tundra and steppe plains. Consequently the Alps and other southern mountain ranges support a diverse biota, which comprises species with varied and complex evolutionary histories. It is not surprising then that the application of molecular phylogeographic methods to alpine regions is generating a kaleidoscope of patterns and possible pathways produced by Quaternary glacial cycles.

For example, the globeflower, *Trollius europaeus*, shows higher genetic diversity (AFLP) in the Alps than the

Pyrenees or northern Fennoscandia. This indicates that the small Pyrenean populations at the southern edge of the range have undergone genetic drift, whereas the Arctic populations were probably the result of a postglacial founder event from the south. The variation among Alpine populations suggests that they are fragmented relics of larger southern refugial populations (Despres *et al.* 2002). By contrast, in a more temperate snail, *Cepaea*, detailed sampling and many allozyme loci showed three major genetic types, stretching east to west along the Pyrenees, probably as a result of post-glacial colonization from different LGM sources (Ochman *et al.* 1983). Eastern Alpine populations of Norway spruce, *Picea abies*, show fairly high mtDNA variability and are close to probable glacial refugia, whereas western Alpine populations are monomorphic, suggesting a postglacial colonization bottleneck and fixation (Gugerli *et al.* 2001). The high Alpine plant *Pritzelago alpina* (3250 m) and the montane *Anthyllis montana* (2700 m) have been compared for their genetic structure (ribosomal ITS, AFLP) through their similar European mountain distributions. Divergence was lower in *P. alpina*, whereas *A. montana* showed subdivision in the Maritime Alps. It is argued that the genomes of these species have been moulded by different range change cycles in glacial and interglacial periods (Kropf *et al.* 2003). A good example of the detail that can be obtained for postglacial colonization events in mountains is seen in the leaf beetle *Goniotena pallida* in the Vosges (Mardulyn 2001). Using highly variable mtDNA CR sequences, a geographical haplotype network indicated the recent founding and expansion of populations from the south to the north of the mountains.

A long-standing debate concerns the 'nunatak' and 'tabula rasa' hypotheses for the respective survival or extinction of species through the LGM in glaciated regions. Recently, genetic data have been adduced both in support and against these possibilities for particular high Alpine or high Arctic species. For example, in the cushion plant *Eritrichium nanum*, cpDNA and AFLPs identify two or three distinct genomic regions in the Alps, and it is argued that the populations survived in nunataks in the western and eastern Alps through the Quaternary glaciations (Stehlik *et al.* 2002). Another high Alpine plant, *Phyteuma globularifolium*, can be divided on AFLPs into four clear genetic groups along the Alps. In this case the identification of peripheral unglaciated siliceous refugia argues for postglacial colonization of the main glaciated body of the Alps, with no need to invoke nunataks (Schonswetter *et al.* 2002). It would seem very difficult to prove or disprove continuous nunatak survival with fossil or genetic evidence. A local population may go extinct for a while under adverse conditions in the LGM, with recolonization from a warm finger valley as the climate improves. And this may be repeated. Only major areas well separated by ice for a long time, like the Canadian High Arctic Islands, would seem capable of yielding any clear genetic signal.

4. THE ARCTIC RING

Until recently, most species' phylogeographies were obtained from temperate regions, so concepts were developed and tested with these. Sampling in the Arctic is not easy, but several Holarctic phylogeographies have been produced, including mammals, birds, plants and crustaceans (Weider & Hobaek 2000; table 2). Given the much colder climate and extent of the Arctic ice sheets at the LGM, the distribution of such species would generally have been pushed to lower latitudes, and this is borne out by fossil records in central Europe, central Asia and North America. However, large areas of northeast Asia and northwest America at these high latitudes were not glaciated and refugia have been proposed on biogeographical grounds with some fossil support, notably in Beringia (Pielou 1991; Abbott & Brochmann 2003). Consequently, some parts of an Arctic species distribution would have been totally displaced, whereas others only expanded or contracted postglacially. This could produce various signatures in the genetic data.

There is phylogeographical structure in all the investigated species, being very marked in small mammals like lemmings and voles, whereas the mobile waterflea and reindeer show less. The ptarmigan and migratory dunlin are intermediate. The major pattern of this division is similar in the high Arctic small mammals and purple saxifrage, with DNA divergence between European and Beringian clades representing 5–10 full glacial cycles on current rate estimates, and with further regional subdivision over fewer oscillations. The true lemming shows distinct parapatric Euro-Asian, Central Asian, Beringian and Canadian clades, which meet near the Lena, Kolyma and MacKenzie Rivers. The collared lemming shows five similarly placed clades, with the Eurasian distribution being further subdivided near the Ural mountains. These mountains also seem to delineate the North European and Central Asian clades of the root vole, with the latter meeting the Beringian clade again at the Kolyma River. In the circumarctic clonal sedge *Carex*, the boundaries of *Ca. lugens* and *Ca. ensifola* are also near the Lena and Kolyma Rivers, which is also an area of high genetic diversity (Stenstrom *et al.* 2001). The Kolyma River and possibly the Urals separate DNA clades in the dunlin, which is remarkable phylogeographical fidelity in this migratory species. Although the genetic divergence within the rock ptarmigan and tundra reindeer is less and probably occurred mostly within the last glacial cycle, there is sign of incipient geographical structure with genotype differences among these regions of the Arctic Ring (figure 2). Such coincidence of boundaries is probably not due to chance, and may reflect common range changes that tend to produce suture zones as in Europe and North America (Hewitt 1996, 1999). A significant number of taxon boundaries in northern birds, butterflies and other groups also coincide with these contacts.

The development of such marked genetic structure over several Ice Ages implies that these regional genomes have survived separately through large range shifts and demographic fluctuations. There has been little if any effective mixing. The genotypes now in the European Arctic probably survived in the extensive tundra of central Europe, and fossils of several species from Late Quaternary have been found there (table 2). They then colonized north and east as it warmed. Similar late glacial fossils have been recorded near the southern Urals, and populations there could have acted as the source for the expansion of genomes across the Russian Arctic. There must also have been East Asian refugia to account for the distinct

Table 2. Terrestrial species with Holarctic ranges showing distinct phylogeographical pattern, with some indication of their putative divergence times derived from DNA divergence, glacial refugia and postglacial expansion. Fossil evidence, where available, is indicated. HA, high Arctic. See references for details.

| species	range	marker	phylogenetic structure	divergence time (Ma)	refugia	expansion	references
Rangifer tarandus, tundra reindeer	Circumarctic, HA fossils, mobile	mtDNA, CR	seven clades 1–2%	0.1–0.3	Beringia-Asia west EurAsia North America	150 kyr 15 kyr ragged	Gravlund *et al.* (1998) Flagstad & Roed (2003)
Lemmus spp., true lemmings	Norway, Baffin Island HA fossils central Europe, central Asia, North America	mtDNA, cytb	four clades 3.8–7.9%	0.5–1.0	Beringia East Asia Siberia North America	expand few haplotypes few haplotypes expand	Fedorov *et al.* (2003)
Dichrostonyx ssp., collared lemmings	White Sea, Greenland, HA, fossils central Europe, Beringia	mtDNA, RFLP	six clades 1–7%	0.1–1.0	Beringia Arctic Islands East Asia central and west Siberia	north to east expand low diversity low diversity	Fedorov & Stenseth (2002)
Microtus oeconomus, root vole	Holland–Alaska HA, fossils central Europe, south Urals	mtDNA, cytb	four clades, 2.0–3.5%	0.2–0.6	Beringia south Urals Caucasus Central Europe	not far few haplotypes north expansion north expansion	Brunhoff *et al.* (2003)
Microtus agrestis, field vole	Gallicia–Baikal, not HA, fossils central Europe, south Urals	mtDNA, cytb	three clades, 0.5–5.2%	0.1–0.6	Beringia south Urals Carpathians Iberia	across Asia north and west expansion not far	Jaarola & Searle (2002)
Lagopus mutus, rock ptarmigan	Circumarctic, HA resident	mtDNA, CR	seven clades, 0.21–1.12	0.05–0.1	multiple, e.g. Greenland Beringia/Aleuts Siberia	several recent expansions 4% diversity within lineages	Holder *et al.* (1999, 2000)
Calidris alpina, dunlin	Circumarctic, summer migrant	mtDNA, CR	five clades, 1.1–3.3%	0.1–0.3	West Africa Arabia southeast Asia Central America	west EurAsia Siberia Beringia Canada	Wennerberg (2001); Wenink *et al.* (1996)
Daphnia pulex, waterflea	Circumarctic, HA clonal, polar waters	mtDNA	seven clades, 0.5–3%	0.2–1.5	Periglacial some more local clones	mixing, but some North American Eurasian difference	Weider *et al.* (1999)
Saxifraga oppositifolia, purple saxifrage	Circumarctic, HA fossils Canada, Alaska, south of Ice, North America and EurAsia	cpDNA	two clades	< 2	Beringia North America EurAsia	high diversity North America low diversity EurAsia	Abbott *et al.* (2000)
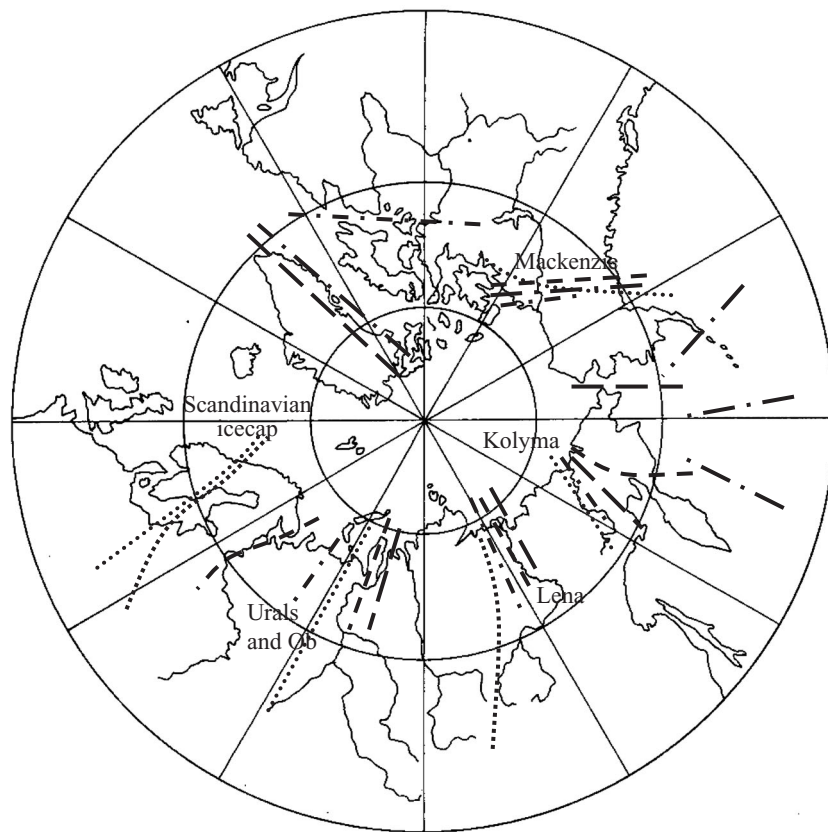
Figure 2. A polar projection of the general regions of contact between diverged DNA clades of six Holarctic species (short dash-dotted lines, collared lemming; short dashed lines, true lemming; circle dotted lines, root vole; square dotted lines, field vole; long dash-dotted lines, ptarmigan; long dashed lines, dunlin). Note the clustering near certain features. The Scandinavian cluster forms where the last remnants of the icecap melted.

genomes of the Lena and Kolyma River regions. Fossils of some species occur in Beringia and it seems likely that these genomes survived there in favourable unglaciated parts.

The range shifts, contractions and expansions that were involved in this Late Quaternary history of Arctic species may be expected to leave some genetic signs of demographic changes, as well as geographical ones. The diversity within many of these clades is relatively shallow or low, indicating contractions and expansions over the last glacial cycle (table 2). For example, the Greenland and Svalbard small-bodied tundra reindeer have low diversity and almost certainly colonized and adapted postglacially. Only 4% of genetic variation occurs within lineages of the ptarmigan, with divergence from the middle of the last Ice Age. In the root vole most clades are shallow, and the Central Asian one contains few haplotypes that range from the Urals to Kolyma River, which suggests extensive postglacial colonization from a small source. Both the true and collared lemmings show little diversity in Asian and Canadian clades. Mismatch analyses indicate an expansion in the Beringian and Canadian clades of true lemmings, perhaps dating from 150 ka after the penultimate glaciation (Illinoian). With the acquisition of larger genetic datasets, the use of automated DNA marker identification and analysis using emerging statistical methods, clearer historical explanations will emerge on these and other Arctic species.

5. BERINGIA

The region called Beringia is about the size of Europe and includes Eastern Siberia and Alaska. Throughout the Quaternary these have been joined by lowered sea-levels during each Ice Age. There is animal and plant fossil evidence for a high tundra mixture of species surviving in unglaciated parts through the last Ice Age. Indeed, a growing number of genetic studies in a range of species describe a distinct phylogenetic clade through the region (table 2; figure 2). The survival of reasonable population numbers through several glacial cycles would be expected to retain higher genetic diversity than those in areas recolonized more recently. Such a pattern is reported in several species, including the purple saxifrage, waterflea, whitefish (Bernatchez & Wilson 1998), the sedge *Carex* (Stenstrom *et al.* 2001) and snow goose *Chen caerulescens* (Quinn 1992). But this signal is not seen in the lemmings and root vole.

Given that Beringia was a major refugium for some Arctic species through recent Ice Ages, the question arises as to its role in the recolonization of surrounding regions. To the west of Beringia, its effect seems limited in several species by parapatry around the Kolyma and Lena Rivers, which probably depends on the survival and arrival of genomes from central or eastern Asian Ice Age refugia in particular species. To the east of Beringia, the vast area once covered by the Cordilleran and Laurentide ice sheets

may have been colonized from several directions, including Beringia, unglaciated parts of the Canadian High Arctic, south of the ice from West and Central USA, and from the east coast of North America. Genetic studies are providing pertinent information on this point for several species.

Thus cpDNA data on the arctic perennial *Dryas integrifolia* combined with a good fossil record indicate that haplotypes from a Beringian refugium spread east and south into the adjoining previously glaciated areas, whereas those in the southeast refugium around New Jersey spread north (Tremblay & Schoen 1999). The genetic data also indicate that an unglaciated High Arctic refugium in the region of Bathurst and Ellesmere Islands also contributed significantly to the southward colonization, although at present fossil evidence is lacking. They also suggest that some colonization may have occurred from the Atlantic coast of the Nearctic. Such postglacial colonization from multiple refugia is also likely in the *Daphnia pulex* complex, where genetic diversity declines eastwards from the Yukon and Bathurst Island and indicates these regions as major contributors to nearctic colonization (Weider & Hobaek 2003). The Beringian haplotypes of the lake whitefish *Coregonus clupeaformis* only colonized a short way south into the adjoining previously glaciated areas, whereas those of the lake trout *Salvelinus namaycush* spread broadly east and south to Baffin Island and the US border (Bernatchez & Wilson 1998). Both these fishes colonized other parts of the deglaciated area from other southern and eastern refugia. Likewise mtDNA haplotypes of the Arctic–Alpine ground beetle *Amara alpina* are richest in Beringia, and colonization across to Hudson Bay saw a reduction in diversity (Reiss *et al.* 1999).

However, in the collared lemming mtDNA phylogeography indicates that haplotypes from the western Arctic Islands colonized eastwards to Baffin Island and Greenland, and those in Beringia did not (Fedorov & Stenseth 2002). In the true lemming it is also argued that the mtDNA haplotypes did not spread out of a Beringian refugium, but that the distinct Arctic Canadian clade colonized postglacially from the south, because there are no fossil records in the High Arctic (Fedorov *et al.* 2003). Also, in the ermine *Mustela erminea* the Beringian mtDNA clade is found only in Alaska and Siberia and meets parapatrically the continental clade in southeast Alaska. This latter probably colonized from the south (Fleming & Cook 2002). There is genetic and fossil evidence of colonization of the nearctic from the south in several other species. So rather like northern Europe, deglaciated areas were variously colonized from different directions by particular species (Hewitt 2000).

Such complex spatial and demographic changes are highlighted by the first palaeogenetic studies in the region on current and permafrost DNA samples of the brown bear (Leonard *et al.* 2000; Barnes *et al.* 2002). The considerable mobility of these animals is reflected in the data, and clade monophyly and haplotype turnover suggest isolation and expansion through the LGM. It is even possible that they went extinct in Alaska from 30 ka and recolonized from East Siberia after 20 kyr. So the Siberian part of Beringia may be a long-term refugium for this species and fossil DNA from here could be informative.

6. NORTH AMERICAN REGIONS

The recent flow of phylogeographical literature, particularly from North America (Avise 2000), precludes detailed reporting here; it shows differences from Europe but also there are clear similarities. Geography and climate determine the distinctive phylogeography of species in different parts of both continents, and North America may also be usefully considered as several principal regions (Hewitt 2000). There are illuminating studies in the Arctic, northwest and southwest regions, which have very different biotas and phylogeographical patterns that have been produced by their distinct geography and climatic changes. The southeast region was where molecular phylogeography began (Avise *et al.* 1987), and shows high species richness and substructure in terrestrial and aquatic organisms (Avise 2000). This was probably generated by survival in refugia through repeated climatically driven range changes, somewhat like southern Europe. This southeast biota also emphasizes that species sharing biogeographic history can exhibit concordance of phylogeographical pattern and produce suture zones (Walker & Avise 1998). The southern Appalachian region appears to have been a refugial area for many species, and populations south of the Laurentide ice sheet have colonized northwards postglacially. Many phylogeographies from this northeast region show signs of this (e.g. Bernatchez & Wilson 1998; Austin *et al.* 2002; Starkey *et al.* 2003), and the effects of Ice Ages and postglacial colonization on the intertidal communities of the northwest Atlantic seaboard are apparent in comparative phylogeographies (Wares 2002). These reveal a transition (suture) zone at *ca.* 42° N, about the latitude to which the ice sheet extended.

(a) *The Pacific northwest coast*

For many species the genetic evidence shows that the previously glaciated regions of North America were colonized from several directions, particularly the south. In particular, and aided by phylogeographical studies, the colonization of the northwest quarter of the continent, which includes Beringia, is becoming clearer. In a now classic study, allozymes and fossil records were combined to reconstruct the colonization of the lodgepole pine, *Pinus contorta*, from south of the ice some 2200 km up the northwest coast to the Yukon (Cwynar & MacDonald 1987). It reached southeast Alaska and the coastal islands by 10 500 BP, but did not reach the inland side of the coast range until 2300 BP. The phylogeography of several species, particularly mammals, argues that this region, where southeast Alaska meets the Yukon and British Columbia, has been colonized from north, south and east; it is something of a tripartite suture zone.

For example, both the longtailed vole, *M. longicaudus*, and the dusky shrew, *Sorex monticolus*, have diverged clades (*ca.* 6% cytb) that colonized from the south, on separate coastal and inland routes. They have other distinct clades to the south in the coastal, Cascade and Rockies ranges, which indicate survival over several Ice Ages, and whence they probably colonized northward, after the LGM (Conroy & Cook 2000; Dembolski & Cook 2001). By contrast, tripartite colonization is deduced in the ermine *Mustela erminea*, with Beringian, coastal island and inland continental clades: one coming from the north and two from the south (Fleming & Cook 2002). However, the

divergence (1.5% cytb) between the island and Beringian clades is less than both from the continental clade (3.5% cytb), which suggests more complex range changes in previous Ice Ages. It will be interesting to see if the island clade representatives can be found down the northwest coast route. Several plant species have distinct cpDNA clades north from Oregon to Alaska, and these could either be southern postglacial colonists or northern glacial survivors (Soltis *et al.* 1997). On present evidence and along with other studies, perhaps the south to north colonization is more likely.

A rather different colonization history is suggested for the forest-dwelling marten *Martes americana* (Stone *et al.* 2002), with the two clades present probably coming from the regions of the Rockies and Appalachians where LGM fossils occur. The coastal clade is currently in the western mountains and up the northwest coast, whereas the continental clade spreads across from the Appalachians to Alaska. Plant fossil data show that the forests spread quickly up the west coast, arriving in southeast Alaska as early as 12 000 BP, whereas the northeastern forests spread rapidly across to the west, but only after the ice had retreated sufficiently after 9000 BP. Several other species including bears and chickadees probably arrived by similar routes (Stone *et al.* 2002).

The high endemism of this region has attracted much attention (Cook *et al.* 2001), with suggestions that it is a refugium and deserving conservation. With genetic evidence this diversity can be seen to be the result of its geographical position and varied structure, with several postglacial colonizations by diverged genomes and rapid local adaptive evolution. This last component is nicely demonstrated by the clear morphological differentiation among species of Queen Charlotte Islands' carabid beetles, which have little or no mtDNA divergence (Clarke *et al.* 2001).

(b) *The west coast*

Although many species colonized up the northwest coast from the general region of the Cascades and northern Rockies, accumulating genetic data reveal that they have distinct clades to the south, forming a Cascade–Sierra transition. There are also divergences between the coastal Cascade and the interior Rocky Mountains. The phylogeographies of this region that demonstrate this have recently been reviewed by Brunsfeld *et al.* (2001). This pattern of divergence applies to a wide range of animal and plant species and indicates the survival of these clades in or near these regions through several Ice Ages including the last. A particularly well-considered example is that of the tailed frog *Ascaphus truei*, which has a mesic forest disjunct range (Nielson *et al.* 2001). It is distributed through the Pacific Coast Mountains from the southeast tip of Alaska (55° N) to north of San Francisco (39° N), and in the Northern Rockies from Idaho and Oregon (43° N) to southern Canada (50° N). It is associated with upland forest streams in the south, and palaeobotanical evidence combined with reasonably dated mtDNA divergence (13% cytb+ND2) places the initial divergence between the coastal and inland clades at the beginning of the Pliocene. Within these major clades are distinct subclades associated with mountain blocks, whose divergence fits within a Quaternary time frame. The main Rocky Mountains clade shows shallow divergence with local haplotypes, which suggests contraction and expansion associated with recent Ice Ages. Such southern montane divergence is reminiscent of that found in southern European mountains, as in Iberian lizards and beetles for example, and for similar reasons (Hewitt 2000). The role this plays in Quaternary speciation is nicely exemplified in grasshopper taxa of Western American sky islands (Knowles 2000).

The mesic forest biome changes at the Cascade–Sierra transition to a warmer, dryer Mediterranean one. California, with its mountain ranges and deserts, lies at a latitude of southern Spain and Morocco, where the Mediterranean Sea has for many species effectively separated Europe from North Africa for 5 Myr. Consequently its phylogeography should also differ. A particular feature of California is its ring of late Pliocene mountain uplift around the hot central valley. South of the Cascades at Mount Shasta the mountains divide into Coast and Sierra ranges, which meet again at Tehachapi some 500 miles south. Among other things these relatively recent uplifts have produced some classic ring species that have been assessed phylogeographically, notably the salamander *Ensatina eschscholtzii* (Moritz *et al.* 1992) and recently the dusky-footed woodrat, *Neotoma fuscipes* (Matocq 2002). These show several clades with divergence beginning in the Pliocene and continuing through the Quaternary. In *Ensatina*, palaeogeography and phylogeography suggest a northern 'oregonensis' genome began to migrate south down both emerging Coast and Sierra ranges some 5 Ma, with range contractions and expansions producing more clades, subspecies and forms. This pattern is still being modified by major geological and climatic changes (Wake 1997). The similar distribution of *Neotoma* shows a somewhat different phylogeography. Its deepest phylogenetic divergence is *ca.* 2 Ma between north and south mtDNA clades, with south and central Sierra clades diverging *ca.* 0.7 Ma. The shallowness and mismatch distributions of several clades reflect recent contractions and expansions, particularly in the northern parts of the range. Some barriers like the Sacramento delta, Sierra river glaciers and Tehachapi mountains may well explain some concordance in racial subdivision between the salamander, woodrat and other species (Matocq 2002).

Further south, into Baja California, Mexico and increasingly drier habitats, similar comparative phylogeography reveals several concordant genetic divergences that are explicable by palaeogeographic events (Maldonado *et al.* 2001). This combination of approaches is both exciting and fruitful.

7. THE TROPICS

Most biodiversity resides in the tropics, but our knowledge of the genetic diversity underlying this is poor compared with temperate regions. There are, however, a few pertinent genetic studies in American and Australian rainforests, which along with reports from Africa and Asia begin to describe a picture of great diversity and historical complexity, often dating from the Pliocene (Hewitt 2000; Moritz *et al.* 2000).

This is exemplified by an adaptive radiation of a North American salamander, *Bolitoglossa*, into tropical middle America, which has been studied in the montane forests of Costa Rica (Garcia-Paris *et al.* 2000). Phylogeographies of birds and freshwater fishes in middle America are also complex with many lineages (Bermingham & Martin 1998). Most of 35 species of small mammals sampled along the Jurua River in Amazonia show a phylogenetic divide coincident with the Iquitos Arch that formed in the Pliocene. Another set of phylogeographical studies from the remnant strip of tropical forest in northeast Queensland involves several birds, reptiles, frogs and a snail (Schneider *et al.* 1998; Hugall *et al.* 2002). There is concordant mtDNA divergence that probably dates from the end of the Pliocene, which is coincident with a narrow region from which rainforest disappeared in the Quaternary Ice Ages.

Bird species from tropical Africa and South America reflect the pattern seen in small mammals and amphibians, with old Pliocene lineages in the lowland forest and mixed old and recently diverged clusters in the mountains. The properties of such tropical mountains as reservoirs and generators of lineages and species (Fjeldsa & Lovett 1997) would seem to derive from their low latitude and topographic variety, providing warm wet habitats through climatic changes and altitudinal range shifts.

8. MAN AND THE ICE AGES

Genetic studies are becoming increasingly prominent in the interpretation of human evolution through the Quaternary, including the effects of climatic oscillations on our range changes from Africa throughout the world (Underhill *et al.* 2000; Hewitt 2001). In particular, evidence from mtDNA and Y-chromosome markers suggests that our genome contains signals of early colonization of Europe from the near east (30–40 ka) and differentiation in the Palaeolithic (Richards *et al.* 2000; Semino *et al.* 2000). So it is interesting to ask how much of European human genomic structure is due to events before the later Neolithic spread of agriculture from the near east (8–9 ka). During the LGM modern European human range was restricted towards the Mediterranean and we would have colonized out from there as it warmed (*ca.* 16 ka). Parts of Europe show distinct genotype frequencies, particularly from east to west (e.g. Rosser *et al.* 2000; Torroni *et al.* 2001), and although some of these may reflect a Neolithic gene flow route, a large proportion could reflect a postglacial colonization pattern like the hedgehog or oak. As more genetic data become available, its combined analysis should clarify the importance of such range changes on our genome.

9. CONCLUSIONS

The use of DNA markers in combination with palaeoclimatic reconstruction is providing much new information on the evolution of species through the climatic oscillations of the Quaternary. It can identify genomic subdivision and its geographical pattern across a species range, and it provides attenuated signals of past demographic and geographical changes.

Recent studies on Arctic species reveal some congruence in the locations of contact zones between their major genomic clades, which indicates common features of their range changes. Some regional genomes show signs of major contraction and expansion through the last Ice Age.

Temperate species generally have less genetic variation in colder latitudes into which they expanded after the last Ice Age, but this depends on their particular niche and geography. Warmer temperate regions contain greater phylogeographical subdivision, arguing for survival and divergence through many Ice Ages; and, in many species, acting as refugial sources for postglacial recolonization. Several common patterns of colonization are apparent in Europe and North America that seem largely dependent on geography and climate. Significantly, a region may be colonized by several species whose genomes come from different refugia. Geographically disjunct divergent clades 'orphan genomes' provide signs of range changes in earlier interglacials.

The extent of genetic divergence between refugial genomes varies among species and is a measure of the time of their separation. Populations in regions where lineages persist through several climatic cycles can accumulate genetic differences and possibly speciate. They are not affected by their relatives that colonize other parts, but are eliminated by climatic reversals.

The few phylogeographical studies in the tropics reveal great genetic diversity, with montane regions containing shallow and deeply diverged lineages often locally distributed. This indicates survival there from the Pliocene, accompanied by local shifts in distribution, adaptation and speciation through Quaternary climatic oscillations.

Clearly the pattern of genetic variation in a species has great potential to inform us on its history and the forces involved, particularly as analytical methods develop and in the light of new fossils and palaeoclimatic advances. These studies may be directed to understand poorly known regions and biomes of importance in managing biodiversity.

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Discussion

A. Lister (*Department of Biology, University College London, London, UK*). Would it be a correct summary of your findings to say that populations could continue to diverge through multiple Milankovitch cycles without being set back to zero every time a new cycle begins? Are the divergences seen on a time-scale of a few hundred thousand years all of subspecific level, or do you have full speciation too?

G. M. Hewitt. Yes, and yes. (i) Populations of a species show various levels of genetic divergence among the southern refugial regions of Europe. Small differences indicate continuous survival there for only a few cycles, like the grasshopper with some 0.8% mtDNA divergence, whereas larger ones, like in the hedgehog with some 12% mtDNA cytb, would require many cycles over a few million years. They are apparently not affected by their relatives in peripheral areas that are colonized and cleared repeatedly by climatic oscillations. (ii) Populations in regions where lineages persist through several climatic cycles can accumulate genetic differences and possibly speciate. There is growing evidence that speciation has been accomplished postglacially under strong divergent selection in certain cases, particularly in fishes, and rapid divergence is seen in insects and reptiles as well. The refugial taxa of the grasshopper *Chorthippus* have been recognized as species and subspecies, though they form a narrow hybrid zone where they meet. The two species of hedgehog *Erinaceus* from the Iberian and Balkan refugia currently form a contact zone down the centre of Europe. There is debate about the possibility of hybridization. The differences at various levels of taxa and the endemism among refugial peninsulas argues for such accumulation of differences and speciation. I think this principle can be seen in other parts of the world, bearing in mind the particular geography and climate.

GLOSSARY

AFLP: amplified fragment length polymorphism
cpDNA: plant chloroplast DNA
CR: control region
ITS: internal transcribed spacer
LGM: last glacial maximum