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Some genetic consequences of ice ages, and their role in divergence and speciation

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Received 22 March 1995, accepted for publication 30 August 1995

The genetic effects of pleistocene ice ages are approached by deduction from paleoenvironmental information, by induction from the genetic structure of populations and species, and by their combination to infer likely consequences. (1) Recent palaeoclimatic information indicate rapid global reversals and changes in ranges of species which would involve elimination with spreading from the edge. Leading edge colonization during a rapid expansion would be leptokurtic and lead to homozygosity and spatial assortment of genomes. In Europe and North America, ice age contractions were into southern refugia, which would promote genome reorganization. (2) The present day genetic structure of species shows frequent geographic subdivision, with parapatric genomes, hybrid zones and suture zones. A survey of recent DNA phylogeographic information supports and extends earlier work. (3) The grasshopper *Chorthappus parallelus* is used to illustrate such data and processes. Its range in Europe is divided on DNA sequences into five parapatric races, with southern genomes showing greater haplotype diversity - probably due to southern mountain blocks acting as refugia and northern expansion reducing diversity. (4) Comparison with other recent studies shows a concordance of such phylogeographic data over pleistocene time scales. (5) The role that ice age range changes may have played in changing adaptations is explored, including the limits of range, rapid change in new invasions and refugial differentiation in a variety of organisms. (6) The effects of these events in causing divergence and speciation are explored using Chorthippus as a paradigm. Repeated contraction and expansion would accumulate genome differences and adaptations, protected from mixing by hybrid zones, and such a composite mode of speciation could apply to many organisms.

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ADDITIONAL KEY WORDS: — range changes – dispersal – refugia – population structure – hybrid zones – phylogeography – adaptation – DNA sequences – biodiversity.

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This paper was originally presented to the Birmingham Linnean Society Symposium on 'The Origins of Species', September 1994

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INTRODUCTION

There are perhaps two ways of approaching the effects of Pleistocene ice ages on the genetics of speciation, and like deduction and induction they are complementary. Firstly, we may ask what can palaeoenvironmental sciences tell us about these climate changes? How was the distribution of fauna and flora affected by them in the Tropical and Temperate regions? How would these changes operate in regions with different topographies, where mountains, plains, lakes and seas are variously distributed? What would all this do to the genetic processes and structure of the species? Secondly, from the other end, we ask what is the genetic structure of populations, races, species and species complexes, both locally and geographically? How diverged are sister species, subspecies and races, and can we date these? How much hybridization and introgression is there between sister taxa, and what are its consequences? What is the power of gene flow among populations? Is there equilibrium locally or broadly? What can recent invasions tell us about the genetic consequences of range changes? What can we learn from the field and laboratory experiments about the rate and limits of adaptation to changed environments? Having gathered such information, the next step is to join the two sets. Are there common points? Do certain historical events predict observed patterns? Can past processes be inferred from current genetic distribution? And then where do we need more data? This treatment will concentrate on Europe and draw mostly on evidence from terrestrial animals, where I have more experience. It will attempt to be more general where this seems appropriate, particularly as much of our information is coming from North America.

THE ICE AGES

Studies of the last 20 years have produced great advances in our understanding of the palaeoclimate, and this continues apace from new data and theories regularly making headlines. Information is gathered from levels of oxygen and carbon isotopes, magnetic and CO_2 measures, animal, vegetable and mineral remains in cores from the seabed, land and ice, and this is providing evermore coherent descriptions and explanations. It is clear that the last 700000 years have been dominated by major ice ages with a roughly 100 kyr cycle interrupted by relatively short warm interglacials such as we enjoy at present. The Milankovitch theory proposes that the pacemaker of this process is the orbital eccentricity of the earth around the sun (Hays, Imbrie & Shackleton, 1976; Gribbin, 1989). This causes major changes in insolation and along with lesser variations in axial tilt $(40\,000\,\mathrm{yr})$ and precession (23 000 yr) produces a complex of climatic oscillations. Going back further in the Pleistocene and into the Pliocene several long records indicate that the ice ages were less intense prior to 700 ky and that the ice sheets in the northern hemisphere began to grow large around 2.4 Myr (Webb & Bartlein, 1992). Thus while the orbital cycles go back beyond 3 Myr, other factors such as tectonic movement and ocean currents must be modifying their effects over longer periods. Furthermore, while the

ice ages have become an increasingly dramatic feature of the Pleistocene, large oscillations in climate producing large changes in flora and fauna can be clearly traced back into the Tertiary.

Our knowledge and understanding are best for the last glacial cycle (~ 135 kyr) and particularly for the progression from the full ice age conditions of 20000 BP to the warm interglacial of the present (e.g. Coope, 1977; Huntley & Birks, 1983; COHMAP members, 1988; Huntley & Webb, 1988; Bartlein & Prentice, 1989; Webb & Bartlein, 1992). The warm Eemian interglacial (135-115 kyr) gave way to colder conditions with more and more water becoming locked up in icesheets until the onset of the present interglacial some 18000 BP. The lesser orbital cycles continued and there were significant climatic fluctuations during this period. Recent results from deep ice cores in Greenland revealed dramatic switches in temperature above the ice sheet during the ice age, and even more surprisingly during the Eemian interglacial (GRIP Project Members, 1993; Dansgaard et al., 1993). It would seem that average temperatures would change by 10-12°C in 5-10 years and last for periods of 70-5000 years; this was very different from the fairly constant conditions during the last 8000 years of the present interglacial. The European pollen record also indicates that there were marked changes in vegetation at various sites during the last interglacial period, which suggests extensive oscillations in climate over a wide area at this time (Tzedakis et al., 1994). Following the Eemian interglacial there is growing evidence for periodic iceberg discharge into the Atlantic every 15-7 kyr during the last iceage, possibly caused by a cycle of build up of ice and collapse into the sea; the so called Bond cycles and Heinrich events (Bond et al., 1993; Lehman, 1993). It is further postulated that such discharges would seriously effect the ocean conveyer circulation of cold water from the North Atlantic and warm water from the Pacific. These events caused large changes in climate which seem to have had effects around the globe; the reports of changes in vegetation in Florida coincident with the later Heinrich events are particularly significant (Grimm et al., 1993).

These climate oscillations will have been expressed differently in various parts of the globe, depending on distance from the equator, ocean position and currents, continental mass and mountain ranges. Thus in the last ice age the massive Laurentide ice sheet covered much of the North America as far south as 40°N, including the Great Lakes, while the Scandinavian ice sheet only reached 52°N covering parts of Britain and northern Europe. The west coast of North America was not so affected by ice and neither was northern Russia as compared with their highly glaciated neighbouring regions. In the southern hemisphere Australia and South America were not subject to extensive glaciation, but their climates clearly were considerably modified.

South of the great northern ice sheets the topography on the two continents is very different. In Europe the mountain ranges of Cantabria, the Pyrenees, Alps, Transylvania and Caucasus all had large ice sheets, while between them and the Northern ice sheet, the plains of Europe were tundra and cold steppe. In North America, on the other hand, with its more southerly extended ice sheet, the forest vegetation was less distant from the ice and access to Mexico and Central America was relatively straightforward. South of Europe, the Mediterranean Sea and then the North African deserts would both have been barriers in the ice ages, while the Mexican deserts were much less extensive at this time. Further south in the tropics, contrary to the stability they were only thought to enjoy, there is growing evidence for considerable changes in climate and vegetation. In Guatemala sites of 'primaeval'

rain forest has been shown to have had xeric species as recently as 11 000 BP and the current vegetation has developed since then (Leyden, 1984). There are differences reported among the estimates of sea surface temperature in the tropics derived from coral, foraminifer and algae sediments around Barbados of -2° C to -5° C at 16 000 BP, while contemporaneous data from mountain vegetation suggests -4° C to -7° C temperature drop, (see Anderson & Webb, 1994). This is clearly an extremely active field with much new data feeding increasingly sophisticated simulation models of climate change over the last glaciation. In considering the effects of the ice ages on any organism or biota, it will be necessary to be appraised of such advances to understand how they affect the particular part of the world where the organisms live, with its peculiarities of topography and climate.

THE LAST ADVANCE

The effects of such climatic changes on the biota are best known from pollen remains in Europe and North America during the last 20000 years, which have seen one of the largest changes from full iceage to full interglacial conditions from 18000 to 6000 BP (e.g. Huntley & Birks, 1983; Huntley & Webb, 1988; Huntley, 1990). Along with various marine data these have allowed convincing models and simulations of climate and plant distribution over this period to be produced (COHMAP members, 1988; Webb & Bartlein, 1992). At the height of the glaciation, plants which now cover North America and Europe were south of the ice wherever suitable conditions occurred for each species. In many cases there were probably small enclaves surviving as refugial populations, as for example in the south of Spain, Italy, the Balkans, Florida or Mexico. As it warmed and the ice retreated, species expanded their ranges across large areas of previously inhospitable terrain. In North America the more extensive Laurentide ice sheet persisted farther south than the Scandinavian ice sheet of northern Europe, and different combinations of grasses, herbs and trees were involved in the very varied conditions of different places. It is clear from the pollen record and stimulation models that each species responded individually to the climatic change, each tracking their particular set of environmental requirements. Some rather different mixtures of species occurred from those of today and communities were not stable (e.g. Webb, 1986; Prentice, 1986).

This great advance north was dramatically reversed in the Younger Dryas for some 1000 years around 10 500 BP. Ocean currents changed, the ice began to readvance, tundra spread to the south of France and in Northern Europe the birches disappeared. When the Atlantic waters warmed again the northern expansion began once more and by 6000 BP the vegetation distribution was broadly similar to the present. The major reversal in climate of the Younger Dryas has been known for sometime, but in the light of our current knowledge it would seem not to be so peculiar; the climate is characterized by nested oscillations and some of these can be rapid, severe and relatively short.

The rate of spread of plant species both before and after the younger Dryas was remarkably rapid (Huntley & Birks, 1983; Bennett, 1986). Maps of radiodated pollen distribution from cores have been used to estimate this parameter for a number of species in different regions; most estimates fall between 50 and 500 m/year, but in Europe pine and hazel were 1500 m/year and alder 2000 m/year for a while. In North America, the beech spread from Tennessee around 16000 BP to reach its

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northern extent in Canada by around 7000 BP, a distance of 1400 km at a rate of 150 m/year on average. At times this rate was much higher (Bennett, 1985). In his seminal work on beetle remains, Coope (1977, 1990) showed that some of these insects dispersed at great rates after the iceage. Species with current Mediterranean distributions reached England by 13 000 BP but did not apparently return after the Younger Dryas; they are sensitive indicators of climatic change (Atkinson *et al.*, 1987), able to track brief switches in conditions by virtue by their dispersal abilities and nutritional tolerance. Unfortunately other insects do not seem to provide such useful remains as the tough coated beetles, but the presence of species on only some rates similar to those in trees. For example, the grasshopper *Chorthippus parallelus* reached England but not Ireland before the channels were flooded, and this requires spreading at 300–500 m/year from its southern Europe refugia (Hewitt, 1990).

Such rapid expansion raises some interesting questions concerning the modes of dispersal and population dynamics of these species, on which some information may be gathered from invasions that have occurred in recorded times. Hengeveld (1989) discusses a number of these, of which the spread of the collared dove from Turkey to cover all of England and western Russia in 80 years is a dramatic example. Equally impressive is the spread of cheatgrass over western North America from British Columbia to Nevada in 30 years (Mack, 1981). As well as providing or denying refuges during the ice age, the topography of each region will affect the postglacial spread of species. For example, Europe and North America are very different in terms of the size, latitude and orientation of mountains plains and waters. The desert latitudes of North America which could form ice age refugia are continuous with the north and the mountains and plains have a roughly NS orientation. While the plains of northern Europe and Russia are separated from the deserts of North Africa and Arabia by mountains and seas running EW, which act as major barriers to dispersal for many species. Thus those species surviving the iceage in southern Spain, Italy, Greece and Turkey would have had the Pyrenees, Alps, Balkans and Caucasus as barriers. Those in North Africa had the Mediterranean Sea to cross as well.

So far we have considered the expansion northward with climatic amelioration, but a warmer climate will also reduce the species' ability to survive in the south of its range. In the Northern hemisphere we can generally expect the range of species, with its band of northern and southern limits, to move northward during warmings and southward during coolings. The distribution of birds provide some clear examples of such range bands (Harrison, 1982), as do a number of insects.

SOME POPULATION AND GENETIC CONSEQUENCES

The primary effect of such climatic changes on a species are major range changes as it tracks its suitable environment. These will have the large size and periodicity of the major interglacials overlaid with the smaller scale and higher frequency of lesser oscillations. The recent ice core data (GRIP Project Members, 1993) emphasize how fast, deep and short some of these switches can be, and the early work on beetles (Atkinson *et al.*, 1987) indicates that some species can respond quickly. Clearly, organisms which are capable of great dispersal and are not dependent on a slower spreading species will track the changes most closely. In as much as all animals

depend on plants directly or indirectly we would expect general correlation of expansion response of animals and suitable vegetation. Furthermore, considering Europe and North America particularly, a sudden major reversal in climate will eliminate most or all species over a large part of their northern range even if it lasts for only 100 years. A rapid rise in temperature will do the same for the southern part of the range. We can therefore envisage the range oscillating N and S, with only 'central' populations surviving, for example (Fig. 1).

The exact extent and shape of the areas populated by the species will depend on the nature and topography of the land and waters. As already mentioned, Europe and North America are different from each other; indeed, any area of the globe has its unique features. For European continental species the dominant features would seem to be the absolute barrier of the Atlantic to the west, the formidable barrier of the Mediterranean Sea to the south, while the deserts and mountains of the Middle East to the Caspian Sea seem to form a considerable divide. To the east of Europe and across Siberia the barriers to many species are less obvious during the interglacial, but these regions would have been inhospitable during the glacial periods. Southern Europe has many mountains ranging from the Pyrenees to the Caucasus and great plains to the North. So during the ice age many species which now range across Europe would have had their refuges in the southern extremities; pollen records show that deciduous forest was limited to south Iberia, Calabria, Greece and the south Balkans, northern Turkey, the Caucasus and the Caspian Sea.



Figure 1. A diagrammatic species range, a stack of ellipses representing large central and smaller marginal populations, that moves north and south with the warm interglacial and cold glacial periods, A–E. Southern populations of the interglacial A produce the central and the northern parts of the distribution during the change to glacial B conditions. The process is reversed for interglacial C and again for glacial D. The range change to interglacial E requires complete recolonisation of the northern ranges probably from the northern populations of glacial D distribution.

When the climate warmed these species would expand from these refugia and spread north rapidly; some species would colonize from just one refugium, others from several. Their genomes may, or may not, mix to various degrees across the plains of middle and northern Europe. Some refugial expansions would encounter mountain or marine barriers, such as the Pyrenees, Alps, Black Sea, Caucasus and Caspian Sea, with various permeabilities. Clearly the dispersal abilities and environmental requirements of each species will differ and so will the details of these range changes. Significantly, the refugial populations in Southern Europe would not have been readily reinforced, if at all, from their south because of the sea, mountain and desert barriers.

The expansion from the south would be rapid and this would mean that long distance dispersants would be able to set up colonies well ahead of the main distribution. These would expand rapidly so that they would dominate the genome of the leading populations. Later migrants would contribute little since they would be entering established populations at carrying capacity with only replacement dynamics; their reproduction will be logistically low while that of the original colonizers would have been exponentially high (Hewitt, 1993; Nichols & Hewitt, 1994). Such a form of spreading from the leading edge will involve a series of bottlenecks for the colonizing genome which will lead to a loss of alleles and a tendency to homozygosity (Nei, Maruyama & Chakraborty, 1975). Such a mode of expansion may be modelled by computer simulation, and the effects of leptokurtic normal and stepping stone dispersal on allele diversity examined (Ibrahim, Nichols & Hewitt, in prep.). A rectangular array of demes was invaded from one fully populated edge by random mating individuals carrying two loci each with two alleles, to compare different dispersal distributions, growth rates and carrying capacities. Most previous studies have used uniform dispersal functions (Endler, 1973; Sokal, Jacquez & Wooten, 1989; Epperson, 1990, 1993) and our uniform and stepping stone dispersal simulations also produce a fragmented patchwork of high and low gene frequency areas, which may persist for hundreds of generations, but which become more and more fragmented with time. The implications of neutral patches and clines showing such relative stability have been discussed by Endler (1977). As dispersal is made more leptokurtic and more long distance dispersal is employed the patches grow larger and persist (Fig. 2).

Whole areas become homozygous at each locus and this increases as the expansion continues. In the simulations shown in Figure 2 expansion has proceeded from left to right across some 30 average dispersal distances and the populations and major patterns are established in well under 100 generations. For many organisms this would fit within a scale of kilometres and operate within the time frame of brief climatic oscillations. Further expansion from the right hand end would draw on population genomes already homozygous at certain loci over a wide area, and the process would increase homozygosity and the range of some genomes and not others; rather like coalescence in reverse (Fig. 3). Because the climate oscillates, such a local advance over some kilometres will be wholly or partly reversed during a general advance; there will be smaller expansions and contractions nested within larger and larger cycles driven by climate. A partial reversal of a local expansion will eliminate many populations so that only a few of the recently established populations survive in locally suitable places. These will retain the genetic pattern previously established, and if bottlenecked will become homozygous at more loci, so that when expansion recommences some of the genome patches would persist and grow.

One might predict that extensive rapid continued expansions would produce considerable homozygosity with derived genomes spread over large areas of the colonized range, while slower expansions would allow more alleles to survive with less genome divergence among populations and areas. These two extremes of expansion — 'pioneer' and 'phalanx' — may well have different consequences at the regional and subcontinental scale. For example, if Europe is colonized rapidly from several southern refugia the genomes of some refugia may spread over much of the area, while under a slower steady expansion all these refugial genomes may spread more equally (Fig. 4). All of these range changes will of course be affected by the local and regional topography, with mountains, lakes, valleys and plains modifying expansions and contractions.

While expansions and contractions will assort near neutral genetic variation spatially, different environmental conditions across the range will select for different genomes. In particular, populations in the southern refugia may well diverge from each other to suit somewhat different habitats, and also because of small population sizes. It has been argued that founder events are not particularly important for speciation (Barton & Charlesworth, 1984), but there is good evidence that the genome and its epistatic interactions are reorganized by population bottlenecks (Goodnight, 1988; Bryant & Meffert 1988; Carson & Wisotzky, 1989; Meffert &



Bryant, 1992). Thus the processes of expansion and contraction in climatically induced range changes should produce considerable genome reorganization, and this may be particularly important in refugia.

Whilst leading edge populations are expanding north across Europe from southern refugia, the mountains of Southern Europe will be providing refugia themselves during the warm interglacial. Some part of the populations will expand up the mountains as the climate warms, but the distance dispersed will be much less for any degree of temperature change. Consequently we would not expect much dispersal bottlenecking and loss of allelic diversity. If there are many separate mountains they may produce different genomes. If the mountain block is large many alleles and genomes may survive, but if a mountain is small then any populations surviving on it may well undergo a series of bottlenecks.



Figure 2. Computer simulations of two alleles at a locus (white and black) during population expansion into new suitable territory. The area is 40×160 demes, and dispersal comes from the left hand end with eight rows of demes at carrying capacity. The space is filled within about 50 generations. A, the result after 100 generations; B, the result after 600 generations. Within each set the top involves leptokurtic dispersal and the bottom a stepping stone dispersal. Large areas where one allele is fixed are produced by leptokurtic dispersal while normal and stepping stone dispersal preserve allelic diversity.

THE PRESENT GEOGRAPHIC GENETIC STRUCTURE OF SPECIES

Most species are confined to continents, and closely related species often occupy different parts of a continent. For example, in Europe this may be a largely northsouth distinction or east-west one. Detailed studies of species complexes and wide spread species have revealed a great deal of geographic subdivision into sibling species, subspecies, races and forms (Hewitt, 1988). Earlier work relied largely on morphological characters, progressively augmented by behavioural, chromosomal and protein data which revealed greater subdivision, with frequently a patchwork pattern of races and genomes. Hybrid zones are found to occur between many of these parapatric taxa; these are relatively narrows regions where the two genomes meet, mate and hybridize. Extensive investigations in recent years have deduced and demonstrated a number of interesting properties of such hybrid zones (e.g. Hewitt, 1975, 1989; Barton & Hewitt, 1985, 1989; Harrison, 1990, 1993). The two parapatric taxa may differ for a variety of characters, but usually there is some reduction in hybrid fitness, i.e. they are tension zones (Key, 1981). This property has important consequences. Firstly, along with individual dispersal and epistasis among genes, it determines the width of the hybrid zone and its strength as a barrier to gene flow. Secondly, a hybrid zone can be trapped in local regions of low density or dispersal, and remain there until major changes in climate and environment occur. As a consequence hybrid zones may well be stable and long-lived, effectively separating genetically the two adjacent races or subspecies. Because hybrid zones



Figure 3. An illustration of the progressive thinning of genotypes 1-12 which expand from the northern edge of a refugium in the South. The genotype in area 7 by chance comes to occupy most of the expanding front as it goes from south to north with long range (leptokurtic) dispersal.

divide races, subspecies and sibling species they have been seen to be involved as stages and signposts in the process of speciation.

It has been noted that in several places in North America, Europe, Australia, South America and Africa a number of hybrid zones between different species appear clustered. Remington (1968) working with the animals and trees of North America described these as "Suture zones of hybrid interaction between recently joined biotas". In particular he noted several associated with major physical and ecological barriers, including the Sierra Nevada/Blue Mountains, the Rocky Mountains, the Texas Plateau, the Florida Border and the Appalachian Mountains. To the east of Europe the Urals may well be a suture zone and there are a number of hybrid zones which run down the centre of Europe with eastern and western sister taxa, e.g. the toad *Bombina*, the crow *Corvus*, the mouse *Mus* and the grass snake *Natrix* are well documented (Barton & Hewitt, 1985).

The advent of molecular techniques for studying genetic diversity, first allozymes, then DNA RFLPs and now sequencing of mitochondrial and nuclear DNA fragments, offers the opportunity to measure effectively the genetic variation across a species range. Furthermore, one may produce DNA phylogenies for the populations, races, subspecies and species, and these may be related to their geographic distribution. Such phylogeographic studies (Avise *et al*, 1987) allow inferences on the biogeographic history and evolution of the populations and taxa, particularly during the Pleistocene. The appendix provides a summary of a number of these recent DNA based phylogenies at and below the species level. Together they



Figure 4. The expansion from a northern edge where areas to the left (1,2) have expanded by normal dispersal and growth while those to the right (11,12) have involved long distance dispersal and establishment.

reveal a range of sequence divergence over the timescale of Pleistocene, general agreement with the rate of the molecular clock, considerable subdivision of species ranges and evidence of range changes and suture zones.

AN INSECT EXAMPLE

The meadow grasshopper Chorthippus parallelus occurs across Europe to Siberia (Reynolds, 1980) and has been a focus of study over the last 10 years, particularly on a hybrid zone in the Pyrenees between two subspecies (e.g. Butlin & Hewitt, 1985; Richie, Butlin & Hewitt, 1989; Hewitt 1993). It may serve as an example and framework for a discussion of this topic. Its current taxonomy recognises subspecies in Iberia, C.p. erythropus, and Greece, C.p. tenuis, with C.p. parallelus across the rest of Europe from Britain to Siberia and Finland to Turkey. As insects go, it is well known. The hybrid zone along the Pyrenees is only a few kilometres wide and the two parapatric taxa differ in morphology, behaviour and chromosomes. They also differ at two allozyme loci out of some 40 tested. Recently this zone has been studied using DNA markers, a nuclear DNA sequence, mtDNA sequence and rDNA sequence. The noncoding nuclear sequence was also used to study 88 populations across Europe (Cooper & Hewitt, 1993; Vasquez, Cooper & Hewitt, 1994; Cooper, Ibrahim & Hewitt, 1995). This shows that C. parallelus is divided into at least five major geographic regions: Iberia, Italy, Greece, Turkey, and all the rest of northern Europe and west Russia (Fig. 5). The average sequence divergence between Spanish and French haplotypes was 2.5% with no evidence of haplotype introgression across the hybrid zone. All the four southern regions-Spain, Italy, Greece and



Figure 5. Europe, showing the putative ice age refugia and possible expansion routes of *C. parallelus* as deduced from DNA sequences from the sample sites shown. Hybrid zones occur in the Pyreenes and Alps.

Turkey—contained a high proportion of unique haplotype sequences indicating that they have distinct genomes, even though they are not all yet described as subspecies. In contrast there is less haplotype diversity across northern Europe and there is little differentiation between these populations and the Balkans.

This has a number of implications when viewed in the light of ice age history. Firstly, it clearly indicates that the hybrid zone in the Pyrenees was formed by expansion from a refugia in the south of Spain, and one in the Balkans which expanded across Europe to colonize from the French side. Secondly this same expansion populated Britain, Scandinavia and western Russia, but not Italy which was colonized from its own refugium in Calabria (Fig 5). As a further consequence, the widespread Balkan-northern European genome should form a hybrid zone with the distinct Italian one in the Alps, and work in progress supports this (Flanagan, pers. comm.). There may well be other hybrid zones in Greece and the southern Balkans, and the Bosphorus may separate the Turkish genomes. Thirdly there is greater genetic variation in the south than the north of the range both in haplotype diversity in populations and in the formation of distinct geographic genomes. Indeed there are indications of regional differences within Spain, Italy and Turkey, but further sampling is needed to pursue this. Fourthly, the extent of the sequence divergence would suggest that the major geographic components of this complex diverged about 0.5 Myr and this is corroborated by mtDNA sequence data (Lunt, Szymura & Hewitt, in prep.). This carries a further implication, that this current genome structure has evolved through several ice ages and climatic oscillations when the range of this species will have changed drastically each time.

HYBRID ZONE BARRIERS

The hybrid zone in the Pyrenees is a moderately strong barrier to geneflow between the French and Spanish genomes and there has been little if any exchange since it formed some 9000 BP when the ice cap melted (Hewitt, 1993), as can be deduced from the relatively narrow cline widths for characters and markers distinguishing them. This means that such hybrid zones may protect the integrity of two genomes until the next ice age reduces the species to its refugia; and this may recur over several ice ages. A number of studies show that the mtDNA of one taxon appears to have introgressed into its neighbour, e.g. Caledia (Marchant, Arnold & Wilkinson, 1988), Mus, (Gyllensten & Wilson, 1987), Thomomys (Patton & Smith, 1994) Clethriomys (Tegelström, 1987), Triturus (Arntzen & Wallis, 1991) (see also Avise, 1994) and are probably the result of bottleneck hybridization and reassortment during colonization perhaps aided by asymmetrical mating. These may well be localized events since the mtDNA difference is coincident with other characters elsewhere in the hybrid zone in several of these cases. However, such events have the potential to produce discordant mitochondrial and nuclear DNA phylogenies emphasizing the need for substantial studies using several types of sequences. In the context of ice age range changes, most such events will be eliminated, but ones occurring in refugial areas may generate new reassorted genomes which then spread over large areas.

SOUTHERN RICHNESS

The lower genetic diversity noted in the north of C. parallelus distribution as compared with the south may have two causes that stem from the range changes brought about by the ice ages. The first could be the tendency for loss of alleles due to population bottlenecking during expansion with the preservation of more diversity in the larger stable populations remaining in the south. The second could be that the populations in the south survive through several glacial cycles by moving up and down mountains which would involve less bottlenecking, and where there are lots of mountains maintain variety in a sort of metapopulation (Fig. 6). A few allozyme studies provide evidence for this loss of diversity with northward spread, notably in eastern Salamanders Plethodon cinereus (Highton & Webster, 1976), Nearctic wild sheep Ovis dalli (Sage & Wolff, 1986) and the lodge pole pine in western North America (Cwynar & MacDonald, 1987). The principle can also be seen in some recent invasions by weedy species such as the North American barnyard grass Echinochloa microstachya, the European rabbit Oryctolagus cuniculus in Australia, and the oak gall wasp Andricus quercuscalicis across Europe (Barrett & Husband, 1990; Barrett & Richardson, 1986; Stone & Sunnucks, 1993). As DNA studies emerge, particularly from North America, some of these are showing a similar pattern suggesting reduced diversity in postglacial expansions (see Appendix for references). The case of the



Figure 6. A S-N cross section through Europe with southern mountains and northern plains. The grasshopper distribution moves up and down the mountains with climatic oscillations (2-3, 4-5) and one refugial genome colonizes all the northern expansion (1,5). Genomic modifications are prevented from mixing by hybrid zones (3,4,5). Genomes persist in southern mountain regions.

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crested newt Triturus cristatus superspecies across Europe is particularly clear (Wallis & Arntzen, 1989), where species involved in northern postglacial expansion were genetically homogenous over much of their range. The lake whitefish Coregonus *clupeaformis* now occupies most of the area that was under the Laurentide ice sheet and putative refugial populations have more mtDNA diversity than those which have undergone extensive colonization (Bernatchez & Dodson, 1991). Another nice example is provided in the eastern woodrat Neotoma floridana (Haves & Harrison, 1992) where the southern mtDNA lineage shows substantial variability compared with the northern and western lineages which probably expanded out from the south-east. In the North American chickadees Parus ssp, the mtDNA of the subspecies involved in the northern expansions has not diverged and is relatively uniform (Gill, Mostrom & Mack, 1993). There are a number of other reports which are suggestive (see appendix) but often these are primarily aimed at other questions and further sampling is required. As with *Chorthippus* several of these studies also show a greater number of lineages, subspecies and species packed parapatrically in the southern parts of the range, close to where the ice age distribution would have been (e.g. Triturus, Neotoma, Parus). If, as has been argued, the northern interglacial expansions are extinguished by a subsequent glacial period then it seems likely that this southern taxon richness is generated by para/allopatric divergence over several ice ages as populations survive by limited movement of their ranges between environmentally suitable locations. Divergence in local allopatry could be protected by hybrid zones as the forms expanded to become parapatric (Hewitt, 1989).

THE TIME OF DIVERGENCE

It is wise to be properly cautious in using DNA sequence divergence to date phylogenic events. Since the calibration of evolutionary rate for higher animal mtDNA at 2% sequence divergence per million years between two lineages for the first few million years (Brown, George & Wilson, 1979), a number of anomalies, problems and explanations have been advanced (see Avise, 1994). There is undoubtedly a wide error attached to any time estimate based on DNA sequence divergence, but used wisely they can provide information, and often it is all that is available. Of the studies listed (see appendix) a number show differences between divergence times estimated from RFLP and sequence data, even in different directions e.g. Apis (Garney, Cornuet & Solignac, 1992), Onychomys (Riddle, Honeycutt & Lee, 1993), Vulpes (Mercure et al., 1993), Osmerus (Taylor & Dodson, 1994). On the other hand the work on cave crickets Dolicopoda (Venanzetti et al 1993) using an island separation dating provides a rate of 1.7-2.3% Myr while an arthropod scale of 2.3% Myr is derived by Brower (1994b). Consequently the divergent events summarized in the list with sequence divergences of up to 6% could well fall in the Pleistocene. It is interesting that this includes many of the subspecific events, while species tend to have somewhat higher values. The Spanish and North European subspecies of *Chorthippus parallelus* with 1% sequence divergence could well have been originally isolated from 500 000 BP when a particularly prolonged period of cold occurred, which may have eliminated the species in all but one refugial region, possibly Turkey. Europe including Spain would then have been repopulated by genomes from this one refugium, which have survived and diverged in its southern refugia over several ice ages since then.

Each organism is different and will require an individual explanation of its phylogeographic data. Some of the parapatric and subspecific divergences reported in the studies listed are remarkably low, e.g. Malaclemys (Lamb & Avise, 1992), Coregonus (Bernatchez & Dodson, 1991), Heliconius (Brower, 1994a), Salmo (Patarnello et al., 1994), indicating that their common ancestors existed in the late Pleistocene and that relatively recent climatic events have been responsible for their present position. Some well studied regions with obviously dramatic Pleistocene history provide easier solutions. Thus where a species currently inhabits areas that were glaciated, or that pollen cores show were clearly too cold and inhospitable, the task is tractable, as witnessed by studies in northern parts of North America and Europe. The SE USA is also proving to be a good theatre for phylogeographic analysis, where knowledge of the rise and fall of sea levels over the Florida peninsula in the last few million years allows sensible explanations of the parapatry between distinct genomes from the Atlantic and Gulf coasts. Several marine and fresh water organisms are concerned, with one bird and one beetle to date (see 1-12 in Appendix). Such general concordance among species for parapatry of genomes ranges argues powerfully for common biogeographic events involving refugia and range changes due to major climatic oscillations in the Pleistocene and late Pliocene. The emergence of this region as a paradigm for such investigations is not only an accident of geography, but also the concerted application of molecular genetic techniques to a range of species (Avise, 1994). The idea of 'suture zones" where two biotas meet, that Remington (1968) proposed for animals and trees, is pleasingly supported by these Atlantic/Gulf coast parapatic divides. It would be most interesting to examine the Atlantic coast of Europe in such detail and also the Pyrenees, the Alps, the Balkans and Caucasus, as indicated by Chorthippus parallelus.

There are as yet few studies which allow a comparison of DNA divergence across racial, specific and generic taxonomic levels in the same group. The studies on cichlids, blackflies and Heliconid butterflies (see Appendix) show that while within and between closely related species the sequence divergence is around 1%, that among cichlid genera is much less than among those of the two insect groups. This could be due to the perception of genera by different taxonomists, or to a real difference in rates of evolution. Obviously more examples are needed.

ADAPTATION OVER THE ICE AGES

A consideration of the adaptation produced in a genome by the fluctuations of the ice ages contains a dilemma, of which palaeoclimatologists using animal and plant proxy material are well aware (e.g. Atkinson *et al.*, 1987; Huntley & Webb, 1988). Did the changes in distribution occur by a stable genome dispersing to occupy new locations for which it is already adapted, or was new territory acquired by adaptation to it with a new genome evolving as a result of this process? The first proposal makes the use of species remains a robust way of reconstructing climate, while the second means that species may expand their ranges without climatic change. The first is supported by the coherence of the palaeoclimatic record that is emerging, and particularly the ability to model past distributions from present species climate tolerances, while the ability of some species to adapt rapidly to new environments is strong evidence for the second. It may be that the genetic changes required to extend

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a species range are difficult and only rarely possible, so that the general relationship between adaptive norms and environment holds over the timescale of an ice age.

Evidence on the speed and form of adaptation to different conditions comes from several sources, however the relevance of it to ice age range expansion is not straightforward. Firstly, the response in the laboratory of Drosophila melanogaster from non-marginal populations to adverse conditions of dessication stress is rapid, but involves lowered metabolic rate, behavioural activity and fecundity. Similar genetic correlations have been found in other animals (Hoffman & Parsons, 1989). At the edge of the species range the stress and perturbation may demand so much metabolic energy that growth and reproduction are reduced to a critical limit. Parsons (1994) has argued that such 'adversity-selection' leads to an evolutionary dead end, and that major new innovations are more likely to occur in disturbed resource-rich habitats. Conditions at higher latitudes show a greater annual range and this would put greater stress on organisms living there, they must be able to tolerate greater changes. This is thought to explain the lower species diversity at higher latitudes and the greater range of species at higher latitudes — Rapoport's Rule. This also appears to apply at higher altitudes (Stevens, 1992). Consequently adaptation to extend a species range northwards appear difficult, and few species manage it. Secondly, there are a number of recent invasions where clear adaptations have occurred. For example, the comborer moth Ostrinia nubilalis was introduced from Europe to USA around 1910 AD and spread down the eastern side of the USA with its diapause response evolving to less harsh conditions (Showers, 1981). Such changes would be necessary, but in reverse, as a species expanded its range after the ice age. However, while some modifications may be genetically possible, the species range may be limited by other characters. Furthermore, a recent invasion may be possible because the organism is entering a suitable environment in a new part of the world, or one that has recently been modified for predators, competitors and hosts, both probably through human action.

Perhaps the effects of the ice age range changes that are most likely to lead to adaptive novelty and divergence are the different conditions and organisms that a species may meet in its various refugia. Thus the climate, physical environment and mixture of species were different in southern Iberia, Greece and other refugia during the last ice age, and most probably previous ones. Furthermore for most European species interglacial times are relatively short, so that for most of the time their distributions would be oscillating in southern Europe in the longer glacial periods. Populations with new adaptations to northern latitudes acquired in interglacials are also likely to be eliminated by the readvance of colder conditions, with more southerly genomes surviving and spreading south where possible. In the case of Chorthippus parallelus it is quite possible that differences between the subspecies evolved in their refugia. For example populations in the Pyrenees of C.p. parallelus, with a Balkan refuge, and C.p. erythropus with an Andalucian or Lusitanian refuge, differ in a variety of characteristics, including song, movement, pheromones, and a range of morphological characters, some of which may well have evolved as adaptations in these refugia. The parapatric taxa of other hybrid zones also differ in various characters which adapt them to distinct lifestyles, e.g. the toad Bombina bombina and B. variegata (Szymura, 1993), the woodpecker Colaptes auratus (Moore & Price, 1993) the pocket gophers Thomomys bottae and T. umbrinus (Patton, 1993) and a number of others (see Barton & Hewitt, 1985, 1989; Harrison 1990).

Such divergence in adaptations may have been acquired rapidly in one ice age, or

over a longer period, and this question may be answered by determining the molecular divergence between two parapatric taxa. This was done for *Bombina* using a number of molecules — allozymes, albumin and mtDNA (see Szymura, 1993) — to give an estimate of 2–7 Myr; this clearly indicates that the divergence of these hybridizing species began before the Pleistocene. A number of other datings using mtDNA indicate a pre-pleistocene origin of divergence, e.g. the toadfish, *Opsanus*, sunfish *Lepomis*, salamaders *Ensatina*, pocket gophers *Thomomys*, newts *Triturus* (see Appendix). For these their divergent genomes must have remained largely separate through many range changes; in 5 Myr there would have been 50 insolation maxima produced by orbital oscillation with an increase in the ice sheets around 2.4 Myr. They have probably sojourned in different refugia in some cycles. Their extended ranges may have hybridized on many occasions, but evidence of this has been lost by range changes extinguishing intermediate populations.

The Chorthippus parallelus subspecies appear to have diverged in just a few ice ages, and there are a number of cases (see Appendix) where the mtDNA divergence indicates a mid to late Pleistocene common ancestral population. Some of these studies demonstrate significant adaptive changes over such relatively short times, e.g. the woodrat Neotoma (nest building), the stickleback Gasterosteus (marine or freshwater), the trout Salmo (morphology and behaviour), and the butterfly Heliconius (mimicry patterns). Some show morphological difference without significant mtDNA sequence divergence, e.g. the beetles Cicindela, the northern chickadees Parus, the sparrow Melospiza and the woodrat Neotoma, which all indicate local differentiation as recent as the last major expansion. Of course the stupendous adaptive divergence in speciation shown by the cichlid fish in the African rift lakes may well be sympatric or microallopatric, but mtDNA measures indicate that it is recent.

EFFECTS ON SPECIATION

If we assume that *Chorthippus parallelus* did emerge from one refugium, possibly in Turkey, to colonize all of Europe some 500 000 BP, it would subsequently have had its range reduced to southern refugia on five occasions by the major ice ages, expanding out again with each major period of warming. Differences would accumulate in the populations inhabiting Iberia, Italy, Greece and the southern Balkans (Fig. 7). During interglacials some populations would survive in the high mountains of these regions and descend to populate the refugia during the cold periods (Fig. 6). The expanding populations would form hybrid zones as their genomes diverged, which would provide partial barriers to gene flow. The northern populations of the refugia would form the colonists for northern Europe and

Figure 7. A stylized map of Europe depicting the expansion, contraction and expansion cycle from some 150 000 BP in the penultimate ice age, through the last interglacial and glacial periods to the present interglacial for *Chorthippus parallelus* (1–6). It shows the Pyrenees, Alps and Carpathians which are barriers to dispersal as lines, and some blocks of high mountains in southern Europe that act as refuges in the warm interglacial today. We do not know when in the Pleistocene the species invaded Europe (molecular data may help), but let us presume that a common genome entered the refugia in Iberia, Italy and the Balkans (1). During the glacial period they diverge, producing genomes 1, 2 & 3. These expand (2) & (3) with a second phase of genomic variations (second digit, e.g. 12) occurring. In the south the species survives on high mountain islands. The subsequence ice age causes contraction into refugia (4), with only those genotypes near reaching them; the rest go extinct. Note that if two genomes have diverged sufficiently for megatively heterotic loci they will form a hybrid zone in the refugium. The process is repeated (5) & (6), with more mutations and adaptations accumulating (third digit, e.g. 12<u>2</u>). Note that hybrid zones develop at the Pyrenees and Alps, separating the genomes, and may occur elsewhere.



populations and genomes behind them to the south would contribute little if anything to this northern expansion. Different environments and cohabiting species in different parts of the range would select different adaptations, and lack of gene flow would allow divergence to accumulate. This could produce a packing of para/ allopatric genomes in southern regions. Such a scenario seems a plausible explanation for the F1 male sterility and positive assortative mating shown by the two morphologically distinct subspecies of *C.p. parallelus* and *C.p. erythropus*, which now hybridize in the Pyrenees. It is not difficult to image that this process could go further to full species — the toad *Bombina* is arguably there. In some organisms the process may well go faster.

This process is difficult to fit into the standard models of speciation. It contains allopatry, microallopatry, bottlenecks and founder-flush, divergent selection, hybridization and the possibility for reinforcement; only pure sympatric speciation is missing. Furthermore, many European species will have had similar histories and in other parts of the world similar principles would apply. Each species group will have its own detailed story which requires individual research and telling. It is worth noting that whilst the reinforcement model of speciation should apply directly to hybrid zones formed by secondary contact with the build up of isolating mechanisms there is no good evidence from hybrid zone studies, and the recent detailed consideration of hybrid zone properties has clarified the narrow conditions under which reinforcement might occur (Butlin 1989).

Whilst sympatric divergence and speciation is not involved in this grasshopper model, the range changes brought about by the ice ages could have promoted it in organisms which have more specific requirements in terms of food, protection or reproduction. A body of work on host specialist insects like the haw fly Rhagoletis pomonella (Bush, 1994) shows how rapidly divergence can occur when a new host appears, in this case the introduction of the apple. Divergence and speciation may occur readily when the feeding, mating and egg laying are closely tied to the host. During separate oscillations species show individual responses and different distributions (e.g. Coope, 1990) and so new potential hosts may present themselves to be colonized with each range change. Having successfully colonized a new host in one part of the host's range with the new race/species may spread through some or all of the rest of it. Thus it is possible that host-race species clusters, such as that of the treehoppers Enchenopa binotata complex (Wood, 1993) could be the result of shifts onto new hosts when a new mixture of tree species was produced by range changes over the recent ice ages. It would be most useful to have DNA sequence divergence measures for this complex. In the spruce budworm species complex mtDNA divergence suggests that changes from spruce to pine hosts have occurred recently on more than one occasion (Sterling & Hickey, 1994). Also the moth Greya suffusca appears to have switched umbellifer hosts recently, differing very little in mtDNA sequence from G. solenobiella (Brown et al., 1994). More generally, laboratory experiments, particularly with *Drosophila*, have shown that divergence selection can produce both pre- and post-zygotic isolation, even with some gene flow, when the traits selected are involved in some way in the mating system (Rice & Hostert, 1993). This may for example involve selection for different spatiotemporal habitats, which also has pleiotropic effects producing assortative mating and a reproductive isolation. Clearly this includes allopatric as well as sympatric populations, and range changes produce a variety of both situations.

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ACKNOWLEDGEMENTS

Obviously many people have contributed to the development of these ideas through published data and personal discussion. I am most grateful to you all. My own work was supported by grants from EU, NERC and SERC.

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| 272 | | | | | | | | | G. 1 | M. I | HEV | WIT | Т | | | | | | | | | | | | |
|----------------|--------------|--------------------------|-------------------------|--------------------------|--------------------------------------|-------------------------|--------------------------|----------------------|---|--------------------------|--------------------|--|------------|------------------------|-------------------------------|--------------------------|---------------------------|--------------|--------------------------|--|----------------------------|-----------------------|--------|-------------------------------------|---|
| | Author | Lamb & Avise, 1992 | Bowen & Avise, 1990 | Avise & Nelson, 1989 | Saunders, Kessler & | Reep & Avise, 1990 | Avise et al., 1987 | Avise, 1992 | Bermingham & Avise, 1986 Avise, 1992 | Bermingham & Avise, 1986 | Avise, 1992 | Bermingham & Avise, 1986 Avise <i>et al.</i> , 1984 | | Avise, 1992 | Bermingham & Avise, 1986 | Powers, 1990 | Bernatchez & Dodson, | 1990, 1991 | O'Reilly et al., 1993 | | Brown, Bechenbach & | Smith, 1993 | | Fajen & Breden, 1992 | Vogler & De Salle, 1993 |
| | Sample size | n=53 | n=29 | n=40 | <i>n</i> =99 | <i>n</i> =212 | n=60 | 30 pops | 17 nons | | 18 pops | 9 DODS | | 18 pops | E/W rivers | 01-11 | 41 pops | n=525 | 12 pages | n=147 | n=27 | 2 rivers | | 6 rivers | $\begin{array}{c} 28 \text{ pops} \\ t n=78 \end{array}$ |
| s substructure | Distribution | Atlantic/Gulf Florida | Atlantic/Gulf | Florida Atlantic/Gulf | Florida Atlantic/Gulf Efection | Atlantic/Gulf | Florida Atlantic/Gulf | Florida SE USA | E/W rivers SF USA | E/W rivers | SE USA | E/W/ rivers SF USA | E/W rivers | SE USA | (-4.1m) N/S ENA | (NI 40°N) | Canada | (Alagash) | WNA | Q. Charlotte Is. | Pacific-N | N. America | | N. Trinidad Watershed | SE USA Atlantic/Gulf coas |
| t on specie | My | 0.05 m | 0.4m | 0.5m | lm | 1.2m | 5.05m | 0.45m | (-0.3m) 3 1m | 2.2m) | 3.15m | (-2.8m) 4 95m | | 4.35m | <u>-</u> | (0.26m) | 0.4 | 0.15 | 1.2m | | <0.3 | | 1 | 0.5<1m | ~2m |
| Aolecular data | % divergence | 0.1% | 0.8% | 1% | 2% | 2.4% | 10.1% | %6 .0 | 6 90% | 2 | 6.3% | 8 5 <i>0</i> % | | 8.7% | 7 haptps | (0.5%) | 1.2% | 0.5% | 2.49% | (2 lineages) | 0.75-1.13% | within rivers | 2 | ut 5% (1%) | 4.7% (2 lineages) |
| APPENDIX: N | Method | RFLP mt 73 sites | RFLP mt | 61 sites FRLP mt | 89 sites RFLP mt | RFLP mt | 00 sites RFLP int | 13 haptps RFLP mt | 13 haptps RFLP mt | 17 haptps | RFLP mt | 32 haptps RFLP mt | | RFLP mt | DEI Dt | 17 enzymes | RFLP mt | 13 enzymes | RFLP mt | 10 enzymes | PCR mt | 462bp 1.2% between | rivers | Control Region II 465hn sequence | PCR mt 656 bp |
| | | 'Diamondback | 'Black Sea bass' | 'Seaside sparrow' | 'Horseshoe crab' | 'American oyster' | 'Toadfish' | 'Bowfin' | 'Snotted sunfish' | | 'Warmouth sunfish' | 'Bhneaill sunfish' | 0 | 'Redear sunfish' | " 'ts. bindulid feb. | (within south) | 'Lake whitefish' | (4 lineages) | 'Stickleback' | (marine and several freshwater forms) | White sturgeon | (1 sp) D-loop | | 'Guppy' (nonulations) | Tiger beetles' (4 ssp) |
| | Organism | 1 Malaclemys terrapin | 2 Centropristis striata | 3 Ammodramus maritimus | 4 Limulus polyphemus | 5 Crassostrea virginica | 6 Opsanus beta | 7 Amia catva | 8. I otiomis trunctatus | annound annound | 9 Lepomis gulosus | 10 I obomis marnchinus | | 11 Lepomis microlophus | 19 Randarhas harmelitan Mirel | COM COMMON MANAGEMENT 21 | 13 Coregonus clubeaformis |) | 14 Gasterosteus aculatus | | 15 Acipenser transmontanus | · | | 16 Poecilia reticulata | 17 Cicindela dorsalis |

| | | | | - LUILLINGU | | | |
|---|---|--|--|-----------------------|----------------------------------|-----------------------------|-------------------------------------|
| Organism | | Method | % divergence | My | Distribution | Sample size | Author |
| 18 Tetragnatha | Spiders (9 sp) | PCR mt 207 bp 12s rRNA | 12-14% 7-9% 1.5% | v | Hawaii and mainland USA | All species | Gillespie, Croom & Palumbi, 1994 |
| 19 Choristoneura | Spruce budworm (5 sp) (2 clades) | PCR mt 1573 bp COL COIL | <1% within, 2.7-2.9% | <0.5-1.45 | N. America | <i>n</i> =10 | Sperling & Hickey, 1994 |
| 20 Osmerus mordax dentex epertanus | 'Smelt' | RFLP mt 15 enzymes PCR mt Cyt b 300 by | 5.7–8.6% 6.6–8.3% | 2.8-4.3m 2.4-2.9 m | Holarctic | 6 locations n=144 (+651) | Taylor & Dodson, 1994 |
| 21 Ensatina eschscholtzii | 'Salamander' (7 ssp) | PCR mt Cytb 681 bp | 0.8–16.2% | 0. <u>4-8</u> m | W. Coast USA | 22 pops n=24 | Moritz, Schneider & Wake, 1992 |
| 22 Xerobates agassizi (123-4-5) herlandieri | 'Gopher tortoise' | RFLP mt 14 enzymes | 4-5.5% 6% | 2–3m 3m | SWNA allo (?para 1:4) allo | <i>n</i> =56 | Lamb, Avise & Gibbons, 1989 |
| 23 Parus atricapillis carolinensis | 'Chickadees' (several sp & ssp) (F&W carolinensis) | RFLP mt 15 enzymes 48-60 sires | 5% (3%) | ~2m >1m | NS NJ-Kansas Tombiehee R | 38 locations n=225 | Gill et al., 1993 |
| 24 Branta canadensis | Canada geese' Canada geese' large bodied small bodied Hawsian | RFLP mt 19 enzymes PCR Bytb 619 hn | 0.15% 1.2% 1.6% | 0.07m 0.6m 0.8m | W. NA Hawaii | 9ssp few | Quinn, Shields & Wilson, 1991 |
| 25 Chen caerulescens | 'Snow goose' 2 clades | PCR mt control region | 6.7% | 3.35m | northern USA, Canada, Russia | <i>n</i> =81 | Quinn, 1992 |
| 26 Canis lupus latrans | 'Wolf' | RFLP mt | 4.2 9 70% | 1-2m | N. America | n=270 n+240 | Lehman <i>et al</i> ., 1991 |
| 27 Onychomys torridus arenicola leucoaster | Grasshopper mice' | RFLP mt 8 enzymes | - 4 9 - 4 - 4 - 4 - 4 - 4 - 4 - 4 - 4 - 4 - 4 | 3m 2m "Prewise" | WNA (paapatric) | n=64 | Riddle & Honeycutt, 1990 |
| 28 Onychomys leucogaster | Grasshopper mice' (2 mt lineages) | RFLP mt ND2-COI (2kb) 18 haplotypes PCR mt Cytb 270 bp | 1.7-2.1% | Е | W. USA | 25 locations n=96 | Riddle <i>et al</i> , 1993 |

APPENDIX: - continued

GENETICS OF ICE AGES

| | | | APPENDIX: - | - continued | | | |
|--|------------------------------------|--|--------------------------|--------------|---------------------------------|-----------------------|---------------------------------|
| Organism | | Method | % divergence | My | Distribution | Sample size | Author |
| 29 Odocoileus virginianus | 'White tailed deer' (5 ssp) | RFLP mt 15 enzyymes 99 sites | 2–2.5% (3% max) | <1.5m | SE USA Florida | 18 pops n=142 | Ellsworth <i>et al.</i> , 1994 |
| 30 Vulpes macrotis | 'Kit fox' (10 ssp) | RFLP mt 17 enzymes | 1.11% | 0.5m | W&SC USA New Mexico | 10 pops | Mercure et al., 1993 |
| vellox | 'Swift fox' | 177 sites PCR Cyt b 800 bp | 0.8% | | | n=250 | |
| 31 Neotoma floridana albigula mizmbus | 'Eastern woodrat' (9 ssp) | RFLP mt 13 enzymes 90 sites | 3.6–5.2% (3 lineages) | ~2m | E&C USA | 33 pops n=114 | Hayes & Harrison, 1992 |
| 32 Thomomys bottae (5 ssp) tournsendii (2 ssp) | 'Western' 'Pocket gophers') | 28 haplotypes PCR mt Cyt b | 4% 2-10% 1-17% | ~7m | SW USA W USA N California | 14 pops n=37 | Patton & Smith, 1994 |
| 33 Peromyscus maniculatus | 'Deermouse' Calif/Central | ogg op RFLP mt 8 enzymes | 4.7% 3% 0.5m | 2.3m 1.5m | W/E NA (wide range) | 25 pops n=135 | Lansman et al., 1983 |
| 34 Magicicada 17/13 | Periodic cicada' | RFLP mt | 2.6% | 1.3m | AK-KY-TN-NC | n=118 | Martin & Simon, 1990 |
| 35 Simulium verustum (4 sp) verecundum (2 sp) vittatum | 'Blackflies' | PCR mt 16s rRNA 460 bp 840 bp | 1.09% 3.46% 5.55% | ∕2m | N. America | 9 locations n=94 | Xiong & Kocher, 1993 |
| 36 <i>Greya</i> (16 ssp) | 'Moth' | PCR mt COI, COII 765 hr | 16% | 8m | N. America | | Brown <i>et al.</i> , 1994 |
| 37 Pissodes strobi (4 sp) | 'Bark weevil' | RFLP mt | 2-16% | 1-8m | N. America | 19 locations n=249 | Boyce, Zwick & Annadro, 1994 |
| 38 Heloconius (42 sp) Neruda | 'Butterflies' | PCR mt COI COII | 0.5–11% | 0.2–5m | C. S. America | n=51 | Brower, 1994a |
| 2. Detectors (1.2. sp.) 39 Heliconius erato | 'Butterfly' 14 races | PCR mt COI, COII | 3.4% | 1.7m | neotropical | n=52 | Brower, 1994b |
| 40 Akodontine tribe (28 sp) | 2 clades 'Murid rodents' | 950 bp PCR mt Cytb 801 bp | 520% | 2.5–10m | . S. America | n=60 | Smith & Patton, 1993 |

| | | | | APPENDIX: - | continued | | | |
|---------|--|---------------------------------------|------------------------------------|-------------------------|---------------|------------------------|------------------------------|---------------------------------|
| Organi | 2 N | | Method | % divergence | My | Distribution | Sample size | Author |
| 41 Trit | urus marmoratus cristatus carnifex | 'Newts' | RFLP mt 11 enzymes | 8% | 4m | NW France | 49 sites | Arntzen & Wallis, 1991 n=210 |
| | dobrogicus karelini | | | 4.5-5.5% | 2–3m | S. Europe | | Wallis & Artzen, 1989 |
| EN) | s within mar, car & kar 4 | -5% divisions) | | | | | | |
| 42 Salı | no trutta | 'Brown trout' (6 ssp) | PCR mt Cytb 249 bp | 0-1.2% | <0.6m | Italy | 13 pops | Patarnello <i>et al.</i> , 1994 |
| | salar | 'Salmon' | 16S rŘNA 343 bp | 2.5% | 1.3m | Ireland | | |
| 43 Doli | chopoda laetitiae (2 ssp) | 'Cave crickets' | RFLP mt | 0.7% | 0.3m | C. Italy | 6 locations | Vananzetti <i>et al.</i> , 1993 |
| | (5 sp) | | 9 enzymes | 14.5% | 7m | | <i>n</i> =66 | |
| 44 Cho | rthippus paallelus | 'Grasshopper' (2 ssp) | PCR mt COI | 1.0% | 0.5m | France/Spain (para) | 10 locations <i>n</i> =46 | Lunt, 1994 |
| | | | 300 bp PCR ncs Cpn I | | | | 33 locations n=111 | Cooper & Hewitt, 1993 |
| | | | 286 bp | 0.4-3.5% | 0.2–2m | | | |
| 45 Bon | vbina bombina | 'Firebellied toad' | RFLP mt | 9.4% | 5m | E/W Europe | <i>n</i> =2141 | Szymura, Spolsky & |
| | variegata | | 17 enzymes | 1 | | (para) | | Uzzel, 1985 |
| 46 Mu | s musculus | 'Housemouse' | RFLP mt | 3.4% | 2m | E/W Europe | n=91 | Ferris et al., 1983 |
| | aomesiicus castaneus | | 11 enzymes | 4.5% | | (para) | | |
| 47 Api: | s mellifera | 'Honey bee' | RFLP mt | 2.7% | 1.3m | Africa | 68 colonies | Garnery et al., 1992 |
| | | mediterranean african caucasian | 16 enzymes PCR 743 bp COI-II | (sequence 1.9%) | | Eurasia | | |
| 48 Spa | lax ehrenbergi | 'Mole rat' /9n-59_54/58_60) | RFLP mt | 2.4% | 1.2m 0.07m | Israel | 18 pops | Nevo & Beiles, 1992 |
| 49 Mel | anochromis auratus | Cichlid mhima' | PCR mt | 0.15% | m62 | (para) I ake Malawi | 14 locatione | Rouser Stauffer Re |
| | heterochromis | | control region | 1.13% | | Africa | n=100 | Kocher, 1994 |
| Pser | udotrophus zebra | | 445 bp | 2.52% | | | | |
| 50 Proc | avia capensis | Rock hyrax (1 sp) | RFLPmt 10 enzymes | 4.2% between clades | 22 | S. Africa | <i>m</i> =55 | Prinsloo & Robinson, 1992 |
| | | | 19 mitotypes | 0.002% within clades | | | | |

A PPFNINIV. GENETICS OF ICE AGES

| | | | APPENDIX: - | - continued | | | |
|---|--------------------------|--|--|-------------------------|---|-----------------------|-------------------------------------|
| Organism | | Method | % divergence | My | Distribution | Sample size | Author |
| 51 Macaca mulatta fuscata cvclobsis | 'Macaques' | RFLP mt 15 enzymes | 3.7% (1.6%) | <2m (0.8m) | Asia Brahmaputra | <i>n</i> =18 | Melnick <i>et al.</i> , 1993 |
| 52 Macaca sinica group | 'Macaques' (5 sp) | 16 enzymes 97 sites | 1.3-8.1% | <2m | Asia | <i>n</i> =14 (+20) | Hoelzer, Hoelzer & Helnick. 1992 |
| 53 Drosophila montium (6 sp) | 'Fruitfly' | RFLP mt 12 enzymes 50 sites | 0.33-7.58% (| 0.16 - 3.79m | Oriental | 10 strains | Pissios & Scouras, 1993 |
| 54 Melmotaeniidae Glossolepis | 'Rainbow fish' (9 sp) | PCR mt 313 bp control regn 351 bp cytb 49 bp tRNA-Pro | 0-17.9% within genera ssp <4% between genera 17 | <6 | Australia N. Guinca | <i>n</i> =26 | Zhu <i>et al</i> , 1994 |
| 55 Petrogale assimilis purpureicollis herberti etc. | 'Rock wallaby' (9 sp) | RFLP mt 15 enzymes 176 bp | 1-5% | 0.5–2m | E. Australia (Fitroy R. Bowan R. etc.,) | 82 pops n=118 | Bee & Close, 1993 |
| | | | | | | | |