Speciation, hybrid zones and phylogeography — or seeing genes in space and time

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Abstract

The origins and development of the study of speciation, hybrid zones and phylogeography are outlined using evolutionary iconography. This traces the ideas in this field from Lamarck and Darwin through to the present as represented in diagrams and figures. A 'tree of trees' summarizes this growth and current vitality. The new facility to use various DNA sequences from nuclear, mitochondrial and chloroplast genomes to determine genetic variation throughout a species range is examined particularly. There is great genomic subdivision across species distributions, which can be interpreted in the light of the recent demonstrations of severe palaeoclimatic oscillations. Refugia and postglacial colonization routes are proposed for several organisms across Europe. The role of geography in speciation through the Pleistocene is considered. These emerging principles and analyses are applied to data available on a variety of organisms in other regions of the world, such as the Arctic, North America and the Tropics, and including the progress of Homo sapiens through the last ice age. Some suggestions are made for future research directions.

Keywords: colonization, DNA sequence, hybrid zones, palaeoclimate, phylogeography, speciation

Received 5 June 2000; revision received 22 July 2000; accepted 29 July 2000

The naming of parts

The formation of new species was amongst the first tasks claimed by early religion for the creator (Genesis Ch. 1), with the first application of taxonomy, the naming of species, being attributed to Adam (Genesis Ch. 2). Speciation remains a big question in science even after some two centuries of enlightened investigation and debate (Darwin 1859). It is a great attractor for enquiring minds, young and old, and myself included. But it does not seem to be a singularity, rather there appear to be many pathways of divergence to specific status. The considerable advances in our thinking are reflected in two books of articles spanning the last decade (Otte & Endler 1989; Howard & Berlocher 1998). Gene flow can be prevented by many changes sympatrically and allopatrically, while the cohesion of a genome may be maintained by reproductive and ecological selection.

Hybridization was clearly a problem for immutable species concepts, and continued to cause disputes really until the 1970s and 1980s, when the growth of work focused on hybrid zones clarified many of the difficulties (Barton & Hewitt 1985; Harrison 1993). Plants exhibit considerable hybridization and reticulate evolution, but many early leading theories were zoologically inclined (Grant 1963; Arnold 1997). Interestingly, Darwin did not have many problems with hybridization, and his views on hybrids and sterility seem surprisingly relevant to more recent discussions of reinforcement and parapatric speciation (Hewitt 1990). They were apparently overlooked by generations of taxonomists and evolutionists. The dissection of species ranges by hybrid zones clearly indicated considerable geographical genomic subdivision, and begged the question of how major climatically induced range changes had put these in place (Hewitt 1975; Hewitt & Barton 1980).

The term 'phylogeography' is only 13 years old (Avise *et al*. 1987) and concerns the geographical distribution of genealogical lineages (Avise 1998). The ability to use DNA sequence variation for phylogeny reconstruction grew through the 1970s, and has exploded in the last decade with the advent of polymerase chain reaction (PCR). The field has been led by the use of mitochondrial DNA (mtDNA) to determine phylogenetic relationships among animal populations, subspecies and species, which may then be plotted on their geographical distribution. Some of

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Dedication: This is dedicated to Bernard John my tutor, who first showed me the light of genetics and shared his joy in words and deeds.

the earliest reports were on small mammals and fishes in south-east USA, which showed that geographically distinct lineages could be of Pliocene origin, while some populations appeared recently derived (Avise 1994). Some lineages formed parapatric contacts and hybrid zones, and the extent of their divergence ranged through the time course of speciation (Hewitt 1996).

This ability to determine DNA sequence across an organism's range and deduce its history from the divergences it shows was a most exciting prospect. It combines several types of data and promises to illuminate many evolutionary questions. If you will, we can now see genes in space and time.

Evolutionary iconography

The depiction in diagrams and figures of how we view population divergence, hybridization and speciation is most illuminating and educative, and it conveys complex ideas and explanations succinctly; evolutionary iconography is a potent art (Fig. 1). As the Renaissance blossomed and scientific enquiry challenged older beliefs, Jean Baptiste Lamarck in 1809 proposed the idea that the Scala Naturae, the immutable and complete ladder of life, could be climbed by evolution from lower to higher forms. But due to scientific, political and social reasons it was not accepted. It was not until 1859 that Charles Darwin, finally prompted by Alfred Wallace, produced 'On the Origin of Species by Natural Selection', which provided strong evidence and an original mechanism for organic evolution. Mendel's contemporary discovery of discrete heritable factors, the 'genes', was not 'rediscovered' until 1900 — an amazing misfortune. Ernst Haeckel, a fan of Darwin, produced his 'Tree of Life' in 1866, which perceived the evolution of organisms through time as literally a branching process. But some later versions suffered from artistic licence.

The palaeontological records of organismal phylogenies, particularly those of vertebrates are represented as knarled trees, with demographic expansions shown as thickened branches and extinctions as stumps (Romer 1945). Basal connections to the trunk are wisely put as dotted lines, but nowadays with molecular data these can be emboldened and possibly completed. One of the classic uses of genetic data to deduce lineages and build a phylogeny was that of Hampton Carson (Carson 1970, 1983) for Hawaiian *Drosophila*, where the polytene chromosome rearrangements produce a network of derived species on the various islands. From this he postulated routes of colonization and speciation as the volcanic islands emerged over some 5 Myr. The Hawaiian Islands are still a very productive arena (Roderick & Gillespie 1998).

As DNA data became available, initially as mitochondrial restriction fragment length polymorphisms (mtRFLPs), haplotype trees and networks were produced, and were used in some of the early phylogeographies to show population divergence for this DNA sequence across a species range (Avise *et al.* 1979). As well as describing genes in space, this contains a time element because mutational sequence changes accumulate over time — but it requires some independent setting of the clock, as is possible for volcanic islands or with fossils. These approaches have been developed in two major ways, with the assessment of phylogeographic pattern and its causes by nested clade analysis of intraspecific trees (Templeton 1998), and the use of minimum spanning and median joining networks of haplotypes, particularly with human mtDNA studies presently (Bandelt *et al.* 1999). The depiction in these spanning networks of the frequency of haplotypes by their circle sizes gives some clue to past demographic changes, which are perhaps more clearly appreciated in nucleotide mismatch analyses (Rogers & Harpending 1992).

DNA sequences have also been used for the phylogeography of volcanic archipelagos like Hawaii and the Canaries; their special spatial simplicity and temporal certainty allows the historical sequence of colonization and speciation to be determined from gene trees (e.g. Shaw 1995, 1996; Juan *et al.* 1997, 1998). Deducing this in continental theatres over the same time frame of a few million years presents a different, and in some ways more difficult challenge. Many large range changes confound the genetic signal, and need to be taken into account (Hewitt 1989; Harrison 1991). Whilst DNA phylogeography can provide tenable population and species histories from the last ice age and up to some 100 kyr, deduction becomes increasingly uncertain further back, and only in particular geographical circumstances does it reveal ancient genome distributions of 1–2 Myr (Santucci *et al.* 1998).

While palaeontologists were properly careful about the timing of separation of two lineages, when producing an organismal phylogeny they often saw the cladogenesis producing two new species as a simple fork. Those concerned with the process of speciation and its experimental investigation saw the fork rather differently, particularly botanists aware of divergence and hybridization over time (Grant 1963). This often extended and complex process was also evident to those working on hybrid zones, where it was apparent that genomes had developed different degrees of divergence and reproductive isolation (Hewitt 1989). Once again the advent of DNA techniques meant that an organism's phylogeny could be based on sequence genealogies, as was recognized by a number of people (e.g. Barton 1988; Pamilo & Nei 1988; Avise 1989; Hey 1994). The simple phylogenetic fork comprises the separation over time of complexly entwined lineages of one population into two separate bundles, rather like plaiting one clump of honeysuckle up both sides of a window. Whilst this realization embraces the disparate concepts of systematic phylogeny and population genetics, it is taking some time

for entrained thought patterns to be overgrown. The embedded concepts of coalescence and lineage sorting (Hudson 1990) clarify the interpretation of the patterns we see in gene trees as populations diverge and speciate (Shaw 1998). Branching population trees composed of serial arrays of males and females passing on alleles over many generations make it all very clear (Avise & Wollenberg 1997). To describe adequately the organismal phylogeny at and below the species level several unlinked gene trees should be produced; for higher level phylogeny one or two may suffice to give a clear picture at his bigger scale. DNA phylogenies are being used as the basis for mapping the evolution of morphological, behavioural and ecological characters (Kambysellis & Craddock 1997; Wells & Henry 1998). It will be nice to produce the gene trees for characters critical in speciation, and follow their pathways.

This 'tree of trees' that has been sketched out shows how the present multitude of concepts, methodologies and approaches have blossomed in the last decade, and literature searches quantify this in publications. This recent radiation stems from several formative studies in the previous decade now coupled with the increased ease of obtaining DNA sequences. There were a few luminaries in the 1970s and earlier, but not for the first time in evolutionary biology, Darwin is the coalescent!

DNA sequences suitable for the Pleistocene

In our quest to see genes in space and time, the current ability to obtain DNA sequences from many individuals across their current range is fuelling many studies, and it facilitates these component approaches to phylogeography and speciation. Animal mtDNA has led the way in this endeavour, due in part to its molecular and inheritance properties, and also to serendipity (Avise 1998). However, because an organism comprises many genes, it is important to produce several different genealogies for each population and species in order to describe adequately the organismal phylogeny as it diverges and speciates through time. A number of other molecules are available and many more are becoming so through various genome projects. However, these are not often employed due to several factors. The rate of divergence of the mtDNA sequence does seem remarkably suitable for divining the genealogy of speciation; as it is fast enough to show population differences across a species range, and slow enough not to saturate with recurrent mutations over a few million years. This, it seems is the time frame of much speciation (Hewitt 1996; Avise *et al.* 1998).

More slowly evolving sequences are required for deeper phylogenetic investigations, and very fast ones for recent population dynamics and divergence (Table 1). The D-loop

Table 1 Rates of evolution of various DNA markers

DNA markers	Organism	Average of divergence (%/Myr*)	Reference
Nucelar DNA			
Nonsynonymous sites	Mammals	0.15	Li (1997)
	Drosophila	0.38	Li (1997)
	Plant (monocot)	0.014	Li (1997)
Synonymous (silent) sites	Mammals	0.7	Li (1997)
	Drosophila	3.12	Li (1997)
	Plant (monocot)	0.114	Li (1997)
Intron	Mammals	0.7	Li (1997); Li & Graur (1991)
Chloroplast DNA			
Nonsynonymous sites	Plant (Angiosperm)	0.004–0.01	Li (1997)
Synonymous (silent) sites	Plant (Angiosperm)	0.024–0.116	Li (1997)
Mitochondrial DNA			
Protein-coding region	Mammals	2.0	Brown *et al.* (1979); Pesole *et al.* (1999)
	Drosophila	2.0	DeSalle *et al.* (1987)
COI	*Alpheus* (shrimps)	1.4	Knowlton & Weigt (1998)
D-loop	Human	14	Horai *et al.* (1995)
	Human	17.5	Tamura & Nei (1993)
	Human	23.6	Stoneking *et al.* (1992)
	Human	260	Howell *et al.* (1998)
	Human	270	Parsons & Holland 1998
Nonsynonymous sites	Plant (Angiosperm)	0.004–0.008	Li (1997)
Synonymous (silent) sites	Plant (Angiosperm)	0.01–0.042	Li (1997)
Microsatellite			
	Human	5.6×10^{-4}	Weber & Wong (1993)
	Drosophila	6.3×10^{-6}	Schug *et al.* (1997)

*Mutation rate of microsatellite DNA is in unit of mutation per locus per gamete per generation.

evolves particularly quickly and is used for many subspecific phylogeographic studies. It has been something of a surprise to find that nuclear gene introns are some 5–10 times slower than the average mtDNA divergence, while chloroplast DNA (cpDNA) is 10 times slower again. This in turn means that the haplotype variation we see today is an accumulation of more or less ancient mutations, so population and close species differences are largely a sorting of these in space and through time. Few uncomplicated nuclear sequences have been described that match the rate of divergence of mtDNA, which is so suitable for intraspecific population studies. Two examples are the anonymous noncoding nuclear marker used in the grasshopper *Chorthippus parallelus* (Cooper & Hewitt 1993; Cooper *et al*. 1995), and the 3′ coding region *Antennipedia*-class homeobox gene in the locust *Schistocerca gregaria* (Zhang & Hewitt 1996; Hewitt 1998). In both cases these had diverged faster than mtDNA sequences; in the locust it was comparable with the AT-rich control region. Short interspersed nuclear elements (SINE) sequences may offer useful fast divergence, but their multicopy dynamics renders them hard to interpret at present. Microsatellites show much higher mutation rates, many alleles and considerable population variation; but these simple-sequence repeats (SSRs) produce allele homoplasy and there is no clear genealogy (Orti *et al*. 1997; van Oppen *et al*. 2000). However, when used with care they can provide valuable insight into phylogeny, speciation and range expansion (Angers & Bernatchez 1998; Beebee & Rowe 2000; Grant *et al*. 2000). For determining population genetic dynamics and phylogeographic divergence in recent times, i.e. Late Pleistocene and Holocene, a combination of sequence (mtDNA, ncDNA) and hypervariable [SSRs, amplified fragment length polymorphisms (AFLPs)] markers would seem necessary. Examples of apparently recent rapid divergence and speciation, as in Rift Lake cichlids, North-west Pacific sticklebacks and Canary Island beetles, are cases in point (Schluter 1998; van Oppen *et al*. 1998; Emerson *et al*. 1999). So whilst in principle multigenic phylogenies are desirable for the understanding of population divergence and speciation, they are not so easy to obtain, and they will not be cheap.

Hybrid zones and geographic subdivision

In 1968 Charles Remington published a major review in which he discussed suture zones of hybrid interaction between recently joined biotas. This has been largely ignored until recently; I stumbled across it in 1975 when writing a review. In this review he identifies six major suture zones and seven minor ones in North America, each one being a region where many hybrid zones from different species are located. These may be seen as interfaces between regional biotas where many diverged genomes met and hybridized after the last ice age (Hewitt 1993). He

also postulated that several zones were recently formed due to human activity. Interestingly, Remington clearly saw reinforcement speciation as a possibility in such secondary contacts, as his delightful diagrams show, and hybrid zones have been the laboratory for much research trying to resolve the debate about the likelihood of this proposed mode of speciation (Butlin 1989, 1998; Howard 1993; Ritchie & Hewitt 1995). It seems that if it is to develop, prezygotic reproductive isolation must do so quickly on secondary contact, and it may be that gamete competition and preference is a worthwhile place to look (Hewitt *et al*. 1989; Howard *et al*. 1998).

The use of DNA markers to describe species geographical subdivision was seminal in the development of the field of phylogeography (Avise 1998), which occurred in southeast USA where one of Remington's terrestrial suture zones was located. This molecular work concerned marine and coastal species initially and revealed contacts between north and south mtDNA lineages around Florida (Avise *et al*. 1987). Parapatric mtDNA clades have since been found in a variety of terrestrial and freshwater species in this region, with fishes and turtles providing striking examples of phylogeographic concordance (Avise 1996; Walker & Avise 1998; Weisrock & Janzen 2000).

It was realized early on that such subspecific parapatric contacts and their hybrid zones could be remarkably stable in position over time (Hewitt 1975, 1979, 1988). They should be trapped in density troughs and unlikely to move far in a landscape of varying suitability, without that is a major ecological or climatic change. The positions of hybrid zones in grasshoppers in the Alps, Kangaroo Island and the Pyrenees argued that this was so. Furthermore, there were several other parapatric contacts in the Alps, Pyrenees and Central Europe described for the most part on morphological characters: Remington (1968) had speculated that a suture zone might exist in Central Europe. There was much debate about whether hybrid zones had originated through primary divergence or secondary contact (Endler 1982). But it seemed clear that hybrid zones in the Alps and along the Pyrenees could not have been there during the last glaciation, and must have only formed approximately 9000 years ago (Hewitt 1985). Because much of Europe was greatly affected by the ice age, this argument would apply to most hybrid zones in Central and Northern Europe, and elsewhere in temperate regions. The question naturally arose, how did they get there? As well as describing genomic subdivision in space, it was necessary to understand range changes through time (Hewitt 1988).

European postglacial colonization

In parallel with the advances in molecular genetics providing DNA markers for the study of subspecific divergence, there has been much progress in palaeoclimatology.
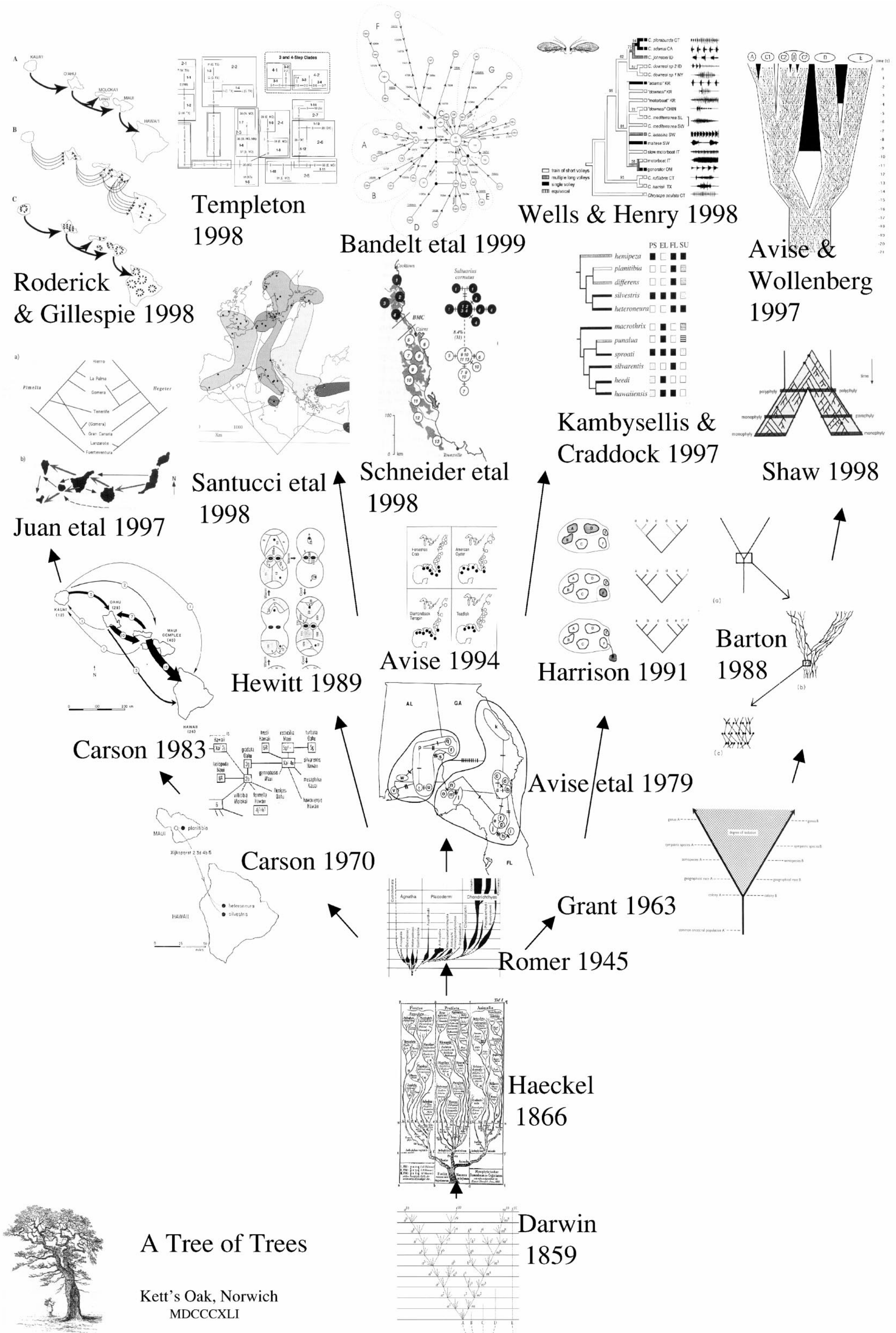
Templeton
1998

Roderick
& Gillespie 1998

Bandelt etal 1999

Wells & Henry 1998

Avise &
Wollenberg
1997

Juan etal 1997

Santucci etal
1998

Schneider etal
1998

Kambysellis &
Craddock 1997

Shaw 1998

Hewitt 1989

Avise 1994

Harrison 1991

Barton
1988

Carson 1983

Avise etal 1979

Carson 1970

Grant 1963

Romer 1945

Haeckel
1866

A Tree of Trees

Kett's Oak, Norwich
MDCCCXLI

Darwin
1859

Fig. 1 A Tree of Trees — to illustrate evolutionary iconography and the development of current ways of seeing genes in space and time. Permissions for the reproduction of these figures have been kindly supplied by the Copyright holders.
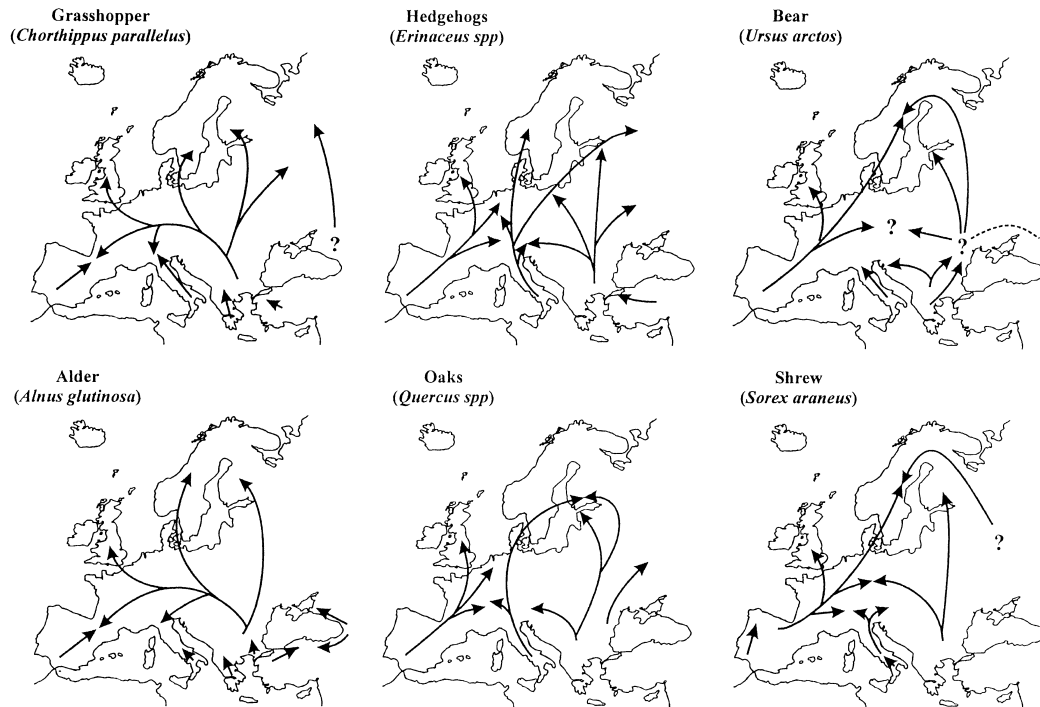
Fig. 2 Three paradigm European postglacial colonization patterns, the grasshopper, the hedgehog and the bear, and three similar species patterns, the alder, the oak and the shrew (see Hewitt 1999 for detailed discussion and references).

Signatures of past conditions come from measures of carbon and oxygen isotopes, radiolarian species, pollen types and other physical and biological residues in cores from sea beds, lake bottoms and ice sheets (Williams *et al.* 1998). They tell of global oscillations in climate with periodic (41 and 100 kyr) ice ages becoming increasingly severe through the last 2.4 Myr — the Quaternary period. A few recent ice and pollen cores manage to reach back over three ice ages to some 400 kyr, and several cover the 125 kyr to the last Eemian interglacial period. These reveal many oscillations within the major 100 kyr cycle, some very severe. Fossil material, particularly pollen and skeletal remains, show that at the height of the last ice age some 20 kyr ago most temperate species, with their main ranges now in Northern and Central Europe, survived in refugia in the south near the Mediterranean. As the climate warmed and the ice retreated they colonized northward.

This colonization was rapid. The Greenland ice cores indicate that interstadial cooling and warming was rapid, with average temperatures falling or rising by several degrees in decades. The pollen core (Huntley & Birks 1983; Bennett 1997) and beetle exoskeleton (Coope 1994) data demonstrate the rapidity of the expansions from southern refugia; some tree species must have spread at over 1 km per year and many others at hundreds of metres per year. Those populations surviving at the northern edge of the refugial area at the end of the cold period would lead the expansion, and long distance dispersers would rapidly fill

the new territories with their progeny (Hewitt 1989, 1993). Such leading edge expansion has been modelled and simulated to demonstrate the effects of such leptokurtic dispersal and founder-flushes in producing loss of alleles and homogeneity over large areas (Ibrahim *et al.* 1996). A corollary of this form of expansion is that it is difficult for populations and genomes behind the front to advance; they must diffuse at a much slower rate, unless they possess some distinct selective advantage that suits a significant change in environment.

While pollen and fossil remains describe these expansions at a generic or specific level, genetic data are required to recognize and understand subspecific events. Molecular markers can identify close similarity between the populations of one genome expansion and hence locate its likely refugium. The recent application of these methods to several species across Europe is quite revealing (Taberlet *et al.* 1998; Hewitt 1999). Three broad patterns of colonization from southern refugia are evident, exemplified by those of the grasshopper, the hedgehog and the bear (Fig. 2). In the meadow grasshopper, *Chorthippus parallelus*, hybrid zones have been described in the Pyrenees (Butlin & Hewitt 1985) and the Alps (Flanagan *et al.* 1999). DNA sequence analysis reveals that these were formed by secondary contact of distinct subspecific genomes emanating from refugia in Iberia, Italy and the Balkans (Cooper & Hewitt 1993; Cooper *et al.* 1995). Other genomes occur in Greece and Turkey, and possibly further east in the Caucasus. The Balkan

Table 2 DNA sequence divergence and estimated maximum time of separation in species groups colonizing Europe after the last ice age. The southern refugia of distinct genomes are given: S = Iberia ; I = Italy; B = Balkans; W = west; E = east. Those not expanding out of their peninsula are in brackets

Organism	DNA sequence	Divergence	Max age (Myr)	Refugia	Authors
Bombina bombina 'fire bellied toad'	mtRFLP	9.4%	5	(I) B B	Szymura *et al.* (1985)
Erinacues europeaus 'hedgehog'	mt cyt b	6–12%	3–6	S I B	Santucci *et al.* (1998)
Triturus cristatus 'crested newts'	mtRFLP	4–8%	2–4	S I B	Wallis & Arntzen (1989)
Arvicola terrestris 'water vole'	mt cyt b	4–7.6%	2–4	S I B	Taberlet *et al.* (1998)
Crocidura suaveolens 'white toothed shrew'	mt cyt b	3–6.4%	1.5–3.2	S (I) B	Taberlet *et al.* (1998)
Mus musculus 'house mouse'	mtRFLP	3.4%	1.7	W & E	Ferris *et al.* (1983)
Microtus agrestis 'field vole'	mtRFLP	2%	1	W & E	Jaarola & Tegelstrom (1995)
Sorex araneus 'red toothed shrew'	mt cyt b	1–3.8%	0.5–2	S (I) B	Taberlet *et al.* (1998)
Ursus arctos 'brown bear'	mt contr reg	2.7–7%	0.35–0.85	S (I) (B)	Taberlet & Bouvet (1994)
Chorthippus parallelus 'meadow grasshopper'	mt 6.7 kb	0.7–0.9%	0.3–0.5	(S) (I) B	Szymura *et al.* (1996)

expansion came to fill most of Europe, probably due to an early start in the east after the ice age and because the Pyrenees and Alps hindered the spread of Iberian and Italian genomes. It seems that a major proportion of postglacial species colonizations followed a similar pattern, including the crested newt, the alder and the beech.

The hedgehogs *Erinaceus europeus/concolor* are parapatric down the centre of Europe, and allozymes and DNA sequences reveal very divergent clades within them in strips down Europe (Santucci *et al.* 1998). This argues for postglacial expansion from several southern refugia, including Spain, Italy and the Balkans, and further genomes occur in the Near East and possibly elsewhere in Mediterranean lands. Oaks and silver fir show similar genome distributions, but this seems a less common pattern of recolonization, probably due to species having refugia south of barriers like the Alps and Pyrenees.

The Pyrenees were apparently less of a barrier than the Alps, as evidenced by colonization patterns like the brown bear, *Ursus arctos*, which from its DNA has colonized Europe from Iberian and Caucasian refuges (Taberlet & Bouvet 1994). Interestingly these expansions met in Central Scandinavia where the last of the ice cap melted some 9 kyr ago and formed a hybrid zone. A number of other mammalian species do the same (Taberlet *et al.* 1998; Jaarola *et al.* 1999), providing another suture zone. This east–west colonizing embrace of Europe may be quite common. For the brown bear, the pattern in central and western Europe has been largely exterminated, but a variety of divergent mtDNA haplotypes occur in southern and eastern parts. This is also true for the shrew, *Sorex araneus*, and other species with this general DNA colonization signal.

Indeed, the presence in species of several genomes and haplotype richness in southern Europe is common, and in contrast to the lower diversity in northern populations. This southern richness is probably the result of refugial persistence and accumulation of variation over several ice ages, while the rapid postglacial colonization is probably responsible for northern purity (Hewitt 1989, 1996). Furthermore, the depth of divergence between lineages in the southern refugial areas indicates that while some species have been there throughout the Pleistocene, others have only been a few ice ages in residence (Hewitt 1999) (Table 2). Clear cases of remnant populations from previous ice ages have recently been analysed in the pond turtle *Emys orbicularis* (Lenk *et al.* 1999) and in the genus *Salamandra* in Europe (Steinfartz *et al.* 2000).

Whilst these three broad patterns of postglacial colonization are now apparent, no doubt there are European species that differ from these (Hewitt 1996). One obvious variant is where a species has only one refugium, as seems to be the case in the natterjack toad (Beebee & Rowe 2000). This has emerged from Iberia up the west of Europe, loosing genetic diversity in the process. Other sets of variant colonization patterns could be produced in species with rather different life histories and ranges from the majority of organisms considered so far. For example, nursing populations of the highly migratory noctule bat from south

and west of Europe have lower genetic diversity than those from central Europe (Petit *et al*. 1999). Such a pattern could be due to colonization from a central forest refugium, and/or that the range has moved north in the Holocene leaving very small southern remnants that consequently became genetically depauperate. Low diversity was noted in remnant populations at the southern edge of the distribution of *C. parallelus* (Cooper *et al*. 1995).

Few freshwater fishes have been examined phylogeographically across Europe other than the brown trout (Bernatchez *et al*. 1992), and now data from chubb and perch are available (Durand *et al*. 1999a; Nesbo *et al*. 1999). These indicate that major river systems such as the Danube are particularly important in colonization patterns of fish, and it should be remembered that glacial meltwater produced greatly modified channels.

More such full range European phylogeographies are needed to understand how Europe's present biota was assembled after the last ice age. Its latitude and geographical structure, with ice age southern refugial peninsulae and northern lands covered in tundra and ice sheets ripe for recolonization, make temperate Europe a relatively simple arena in which to deduce such Pleistocene range changes and their genetic consequences. In many other parts of the world it would seem not so straightforward, although considerable progress is now being made.

Arctic survival — life on the edge

Species living in Arctic and Boreal regions may have had rather different range changes from more temperate ones through the major climatic shifts. Many could have had larger distributions across the large steppe and tundra expanses that covered Europe in the ice age, and the warm Holocene may be a time of population reduction. In an arctic saxifrage, *Saxifraga oppositifolia*, Siberian lemming, *Lemmus sibiricus*, and collared lemming, *Dicrostonyx torquatus*, there is no difference in genetic diversity in previously glaciated and nonglaciated regions (Gabrielsen *et al*. 1997; Federov *et al*. 1999a,b). Likewise, low regional genetic diversity in the nearctic collared lemming, *D. groenlandicus* argues for Holocene bottlenecks (Ehrich *et al*. 2000). There are a growing number of molecular phylogeographies that demonstrate recolonization of Boreal regions from Beringian and other refugia north of the major ice sheets, including fishes (Bernatchez & Wilson 1998), beetles (Ashworth 1997), a classic arctic plant *Dryas integrifolia* (Tremblay & Schoen 1999) and nearctic collared lemming (Ehrich *et al*. 2000). Brown bears also expanded from a Beringian refugium and today have less genetic diversity than 36 kyr ago, as demonstrated from permafrost preserved specimens (Leonard *et al*. 2000).

These high northern latitudes were also colonized from the south when the ice retreated, as demonstrated through a number of phylogeographies. An ensuing loss of genetic diversity has been clearly demonstrated in a range of species, including many fish species (Bernatchez & Wilson 1998). The Pacific north-west of America has produced a number of concordant studies in plants and animals that have expanded north-west from refugia south of the Cordilleran ice sheet (Cwynar & MacDonald 1987; Soltis *et al*. 1997; Conroy & Cook 2000) and show reduced genetic diversity with expansion.

Southern comfort — behind the front

In southern Europe and south-east USA molecular phylogeographies reveal that species contain divergent genomes and are often greatly subdivided geographically, in marked contrast to northward expansions (cf. Hayes & Harrison 1992; Avise 1994; Zink 1997; Bernatchez & Wilson 1998; Walker & Avise 1998; Hewitt 1999; Weisrock & Janzen 2000). This is probably because these regions provided refuges through climatic contractions and expansions over the ice ages, so that genomes survived and diverged without large geographical displacement. Populations could, for example, descend and ascend mountains and rivers to track a suitable environment. Unravelling the spatial genetic history of species in such regions is more complicated than for expansions further north.

This task is greatly helped by the development of the nested clade analysis of haplotype phylogeny and geographical distribution, which was originally used to examine phenotype and genotype relationships for the Adh locus (Templeton *et al*. 1987, 1995). It can detect significant signals of gene flow, range fragmentation and colonization, and when tested against known cases of postglacial range expansion by salamanders, fish, grasshoppers and mice in central USA, it performs well (Templeton 1998). For many organisms, southern populations and genomes in Iberia, Italy, Greece and Turkey did not expand out in the Holocene; more northerly ones did so first. The same is so for southern USA from Florida to California. Consequently the application of nested clade analysis to these situations will be useful. In addition to the aforementioned cases, it has been recently tried in greek chub (Durand *et al*. 1999b) and spanish beetles (Gomez-Zurita *et al*. 2000) to clarify the various range changes and divergences that have occurred through the Pleistocene. Such methods may be rewarding with other species in these regions, including classics such as *Chorthippus* (Cooper *et al*. 1995), *Neotoma* (Hayes & Harrison 1992) and *Ensatina* (Wake 1997).

Regional challenges

Most of our knowledge of how genes have survived in space and time comes from Europe and North America,

and yet most biodiversity resides in the Tropics. Between and among the temperate and tropical zones there are deserts and oceans, about which we have even less phylogeographic information. These regions provide a considerable challenge.

For the Tropics of Africa, Amazonia, Australia, south-east Asia and Central America we have several pertinent studies. Phylogeographies of freshwater fish and birds in Central America are complex with overlaid cycles of colonization from the Miocene onwards (Bermingham & Martin 1998), while in Amazonia the phylogeographies of small mammal species indicate Pliocene divergence for many of them (Da Silva & Patton 1998). Likewise in salamanders from Costa Rica there is deep genetic divergence over short geographical distances, with distinct genomes confined to small areas and elevation zones (Garcia-Paris *et al.* 2000).

Bird species in South American and African tropical forests also show considerable age with 6 Myr or more divergence, while tropical mountains contain both recent and older lineages (Fjeldsa 1994; Roy 1997; Garcia-Moreno *et al.* 1999). This suggests that these mountains are centres of speciation because they are able to retain populations through climatic changes.

The phylogeographies of birds, frogs and reptiles from a strip of rainforest in north-east Australia show evidence of population contractions and a concordant divergence between northern and southern areas that could also date to the end of the Pliocene (Schneider *et al.* 1998). On the other side of the Wallace Line, the lands in and around the Sunda Shelf contain organisms with considerable subspecific divergence, and a number of new mammalian species are being discovered (Surridge *et al.* 1999).

The drier climate of the ice ages caused reduction of the tropical forests and expansion of savannah, and its associated species. DNA phylogeographies of several African bovids indicate earlier reductions and colonizations caused by the Pleistocene climatic changes (Arctander *et al.* 1999). As generators and retainers of much genetic divergence and speciation, it is most important that these tropical regions are studied phylogeographically, to at least provide an understanding of the processes providing this biodiversity. As within Europe and North America, it seems useful to consider regional biotas because these are different and defined by distinct geography and climatic history.

Man through the ice ages

Archaeological studies place the origin of our genus and species in Africa, and these are now being supported by DNA sequence data (Stringer 1996; Harpending *et al.* 1998). Furthermore this evolution was occurring during the Pleistocene and its recent ice ages. *Homo erectus* remains and artefacts occur over Africa and Eurasia from some 1.5 Ma, and those of Neanderthals in Europe from around 300 kyr to 28 kyr ago, when they were finally replaced by *Homo sapiens*, modern man. Two geographically disjunct Neanderthal fossil bone samples have provided similar mtDNA sequences that are distinct from all modern human sequences; this makes the possibility of a Neanderthal contribution to our gene pool quite remote (Krings *et al.* 1997; Ovchinnikov *et al.* 2000).

The bones and tools of archaic forms of *Homo sapiens* are found in Africa from some 500 kyr and apparently developed there into modern man around 200–100 kyr ago. Modern man entered Europe around 41 kyr and Neanderthals declined and disappeared. Data from mtDNA, Y chromosome, ZFX intron, B-globin, HLA, Alu-insertion and microsatellites suggest that the genetic diversity in human populations today reflects passage through a 'long bottleneck' in Africa. This was perhaps as low as 10 000 from mismatch analysis, and expanded between 100 kyr and 50 kyr, and again in Europe about 21 kyr (Harpending *et al.* 1998; Jorde *et al.* 1998). Of course these are not the only possibilities (Templeton 1997).

This long bottleneck may be explained by the gigantic eruption of Mt Toba in Sumatra around 71 kyr, which produced extremely cold weather for perhaps 1000 years as reflected in the Greenland ice core (Ambrose 1998). A likely scenario is that modern man expanded after this volcanic winter from a tropical refugium around 55 kyr, progressed to north-east Africa, then into the Levant and into Europe around 41 kyr ago. Neanderthals were displaced from the Levant and ultimately from Europe by this expansion (Tchernov 1998).

The bones and characteristic artefacts of modern man occur progressively across southern Europe from this time onwards, although those of Neanderthal are still found in France, Iberia and the Caucasus until 28 kyr ago (Mellars 1998). Between his arrival and the glacial maximum some 24–20 kyr modern man's artefacts and remains occur over northern Europe, indicating that he expanded from the south in the warmer interludes. He was probably pushed to the far south of Europe at the height of the ice age. As conditions improved after this around 16 kyr he expanded north again. Pollen and beetle remains show that much of Europe was quite warm by 13 kyr, but this warming was severely reversed in the Younger Dryas from 12 500 to 11 500 years. This cold period greatly affected the flora and fauna of Europe and saw some readvances of the glaciers (Birks & Ammann 2000), and no doubt it affected man.

After this reversal came the Holocene warming, and then the Neolithic agricultural revolution around 9000 years from the Near East across Europe. Classical human gene frequency clines have been correlated with this advance (Cavalli-Sforza *et al.* 1994), as have Y-chromosome markers with Irish surnames (Hill *et al.* 2000)! But recent mtDNA

analyses reveal several diverged lineages that are possibly older and from the period before the Younger Dryas (Richards *et al*. 1996, 1998). The expansions and contractions between 18 and 12 kyr may well have left their mark in parts of our genome.

Conclusion

It is customary in such reviews to make some prospective statement with advice for where we should go from here. As I have tried to convey, this field is so alive and exciting that a young researcher could pick up and run with any one of the questions raised, particularly in the open field of lesser known regions.

Islands, both in the ocean and the sky, provide suitable natural laboratories for productive research on speciation, and so do hybrid zones.

The burgeoning genome projects and advances in molecular technology should provide a vast array of sequences with which to understand how particular genes effect the development of a character, and hence how adaptations evolve. For example, the describing of floral structure, insect song, host shift or sperm preference in terms of particular gene sequences is a possibility. To then understand how this evolved will require placing these results in a spatial and temporal framework, i.e. gene and character phylogeography. This will require further development of powerful inclusive analytical methods, like current coalescent and nested cladistic theory, networks and Bayesian approaches.

More full species phylogeographies are needed from which to deduce colonization patterns, and if these are congruent in particular geographical regions. This will suggest which physical and biological features are more important in determining species history over the last few million years. For example, the challenges faced by organisms in the High Arctic and Wet Tropics are very different, and so too may be their evolutionary responses.

The marine realm is poorly understood, and yet it covers much of the earth's surface. Indeed it is difficult to study, but can provide great resources for mankind. The Tropics contain most of the globe's species biodiversity, yet the extent and pattern of the genetic divergence that underlies this are largely unknown. They are probably immense and complex.

Mankind's growing success places other species under threat. If we are to manage the biosphere sensibly, an understanding of the genetic diversity of different regions of the world and how it evolved is essential. The Quaternary period has seen many changes in global climate, and understanding its effects will prepare us for the future.

Orbital Consequences

The sun and the earth describe orbital changes
 which drive climate cycles and modify ranges.
The shape of the land forms a number of places
 that allow the survival of different races.
When enclaves advance with the ice in retreat
 some form hybrid zones where two ranges meet.
Such regions are common and not very wide
 so the mixing of genes affects neither side.
They divide up the range in a patchwork of pieces
 with echoes and glimpses on the nature of species.
A brief rendez-vous and the ice comes again.

When the glaciers melt so that ranges expand
 some plants will spread quickly where there's suitable land.
Those insects which eat them will follow this lead
 some flying, some walking to establish their breed.
Those that try later meet a resident band,
 they must somehow be better to make their own stand.
But the mixture will change as more types arrive
 and warming conditions allow new species to thrive.
Some will move on to fresh places ahead,
 those that remain must adapt, or are dead.
And then the tide turns and the ice comes again.

Each refuge could foster a deviant form,
 new neighbours, chance changes and drift from the norm.
When the warm breakout comes, those few in the van
 disperse from the edge and breed where they can.
Pioneer pockets grow to large populations,
 a very good place to strike new variations.
Some may not work well with their parental kind
 so stopping the spread of those from behind.
Continental theatres provide plenty of chances
 to establish new morphs in both retreats and advances.
New species may form when the ice comes again.

So what will you do when the ice comes again?
 It could be quite quick, if the ice cores speak plain.
The great ocean currents that warm our green spring
 may stop in a season should the salt balance swing.
Great civilizations in north temperate lands
 must migrate south to the sun and the sands.
But past pollen and dust tell us these will be drier,
 wet forests will shrink and population grow higher.
Our forebears hung on near a sea or a cave.
 They fished and they painted, they dreamed, they were brave.
So like Noah and Eric, we must adapt and survive.

G. M. Hewitt

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