1 *Introduction*

When considering the natural world, it is impossible not to be astounded at the extraordinary diversity of species it contains, and such feelings can only be magnified by the further realisation that what we are seeing is merely a 'snapshot' of the four thousand million year history of life on this planet. Understanding the generation of such a complex situation seems almost totally beyond comprehension, and, indeed, in many respects it is; but, like wonder and astonishment, curiosity is also a fundamental human trait, and through the efforts of many remarkable individuals, significant insight into the source and development of this diversity has been achieved.

Most notable was the discovery by the young Charles Darwin, travelling as a biologist on the *Beagle* in the early 1830s, that all species are related by common descent, and that the vast diversity observed is simply a product of the accumulation of small, but favourable, modifications over enormous periods of time. However, there remained the problem of explaining the inheritance of these modifications, since any form of inheritance where features in offspring are some kind of average of parental features would simply lead to dilution and eventual loss of favourable mutations. A solution was eventually provided by the work of the Austrian monk Gregor Mendel. In the 1860s, Mendel studied the inheritance of various external features of the pea plant in an environment of controlled cross-pollination, and was able to show that the inheritance of characters proceeded in a discrete fashion, with some characters dominant and some recessive. This work, rediscovered in 1900, laid the foundations for the field of genetics, and when fully integrated with evolutionary theory by Fisher, Haldane and Wright in 1930, in what is known as the *modern synthesis*, the major gap in Darwin's theory was filled. Since this time, the theory of evolution has provided a solid framework for understanding the generation of the diversity of species, and continues to grow in strength as the primary unifying thread in modern biology.

1.1 Phylogenetics and human origins

Darwin's reluctance to draw the conclusions from his theory relevant to human origins publicly is well known; his only mention of human ancestry in

The Origin of Species (1859) is a single sentence. But even this brief mention was sufficient to spark substantial conflict with those who saw his theory as an affront to orthodox religious belief. Fortunately, Darwin had an able assistant in his 'bulldog', Thomas Huxley, whose work, *Evidence as to Man's Place in Nature* (1863), confronted the issue of human origins head on.

The Swedish naturalist Linnaeus had already classified humans in the order Primates in the eighteenth century, but it was the work of Darwin, Huxley and others that led to the recognition that the similarities between humans and the so-called great apes – gorillas, chimpanzees, and orangutans – indicated common ancestry; most probably fairly recent common ancestry. Within an evolutionary framework of common descent, the problem of determining relatedness becomes one of constructing a *phylogeny*, i.e. a hypothesised evolutionary history, showing ancestry and branching at those points where mutation has led to a new species.

The *morphological* approach to determining a phylogeny is based on the study of particular physical characteristics of individuals across related species, e.g. size, shape and number of teeth, various body structures and bone lengths, and attempting to deduce the pattern of *speciation* events required to describe the observed differences. A particular strength of this approach is that fossil data, when available, can be included directly. In general, human–ape (i.e. hominoid) phylogenies so constructed had the great apes as one group (family Pongidae), and humans as a sister taxon (family Hominidae) (Oxnard, 1997; Simpson, 1945).[1]

It wasn't until the 1930s that an understanding of the biological mechanism of Mendelian inheritance began to emerge, culminating in 1951 when James Watson and Francis Crick demonstrated the double-helix structure of the DNA molecule, explaining at the same time the manner of its replication, and the way in which errors in this replication naturally lead to mutations. The amazing nature of DNA is apparent in many respects: it is self-replicating, it uses a genetic code that is essentially identical across *all* species, and it carries not only all the coding necessary for the development of a particular individual, but also a record of the evolutionary history of the species of that individual. Theoretical and practical advances in molecular genetics in recent years have allowed unprecedented access to this genetic information, and more and more is able to be read by using a variety of direct and indirect methods.

Early applications of these new approaches to the study of hominoid evolution were by Goodman in the early 1960s (Goodman, 1995), and, in particular,

[1] In the light of later developments, it is interesting to note that Huxley (1863) and Darwin (1871) both considered humans most closely related to the African apes (the gorilla and chimpanzee); see Mann and Weiss (1996) for a historical overview.

by Sarich and Wilson in 1967 (Sarich and Wilson, 1967). Sarich and Wilson employed an immunological technique, measuring the cross-reaction of antigens and antibodies from different hominoid species, as a method of comparing amino acid sequences, the degree of cross-reaction being a measure of similarity. The immune system is obviously highly important in natural selection, and therefore the results obtained by using this method are strongly correlated with the evolution of the species being studied. Results from this new research revealed the fact that humans, chimpanzees and gorillas are in fact more closely related to each other than any of them is to orangutans, so a more accurate phylogeny groups humans, gorillas and chimpanzees (the African apes) together, with orangutans as a sister taxon (see Figure 1.1). The ground-breaking aspect of this work was the imposition of a time scale, leading to an estimate of the time of the human–chimpanzee common ancestor of around 5 million years ago, far more recent than was being indicated by other work at the time.

Other molecular methods include direct sequencing of DNA, comparison of DNA sequences by using DNA–DNA hybridisation (Ruvolo, 1996, 1997), sequencing of various proteins, e.g. fibrinopeptides, haemoglobins and myoglobins (Jones *et al*., 1991), and comparison of the number, shape and banding patterns of chromosomes, i.e. the *karyotype* (Jones *et al*., 1991). These have all revealed a similar picture, although there are inconsistencies with respect to the order of the human–gorilla–chimpanzee split, and placing the orangutan with the African apes, or with the other Asian apes (i.e. the gibbon and siamang). Whole-organism morphological studies, and some recent soft-tissue morphological studies, have since been shown to agree with the molecular consensus (Collard and Wood, 2000; Oxnard, 1997).

All molecular approaches rely on direct access to an organism's DNA or living cells, and therefore, except in rare cases, cannot be applied to fossil species.

By classifying particular morphological features as *characters*, or by working directly with discrete genetic data, a formal approach to the study of evolutionary relationships is possible by using *cladistics* (Hennig, 1966), a method of study that provides a rigorous framework for the construction of phylogenies. Given a set of species, a set of characters are defined by which these species are to be compared, and an *outgroup* species identified, i.e. a closely related species, outside of the group and equally related to all members of the group. Character states that are present in the common ancestor of the group are classified as *primitive*, and do not provide any means of discrimination within the group. Identification of these states is based on the nature of the outgroup species, available fossil evidence, and their simplicity and commonality. Character states that are a result of change within the group are

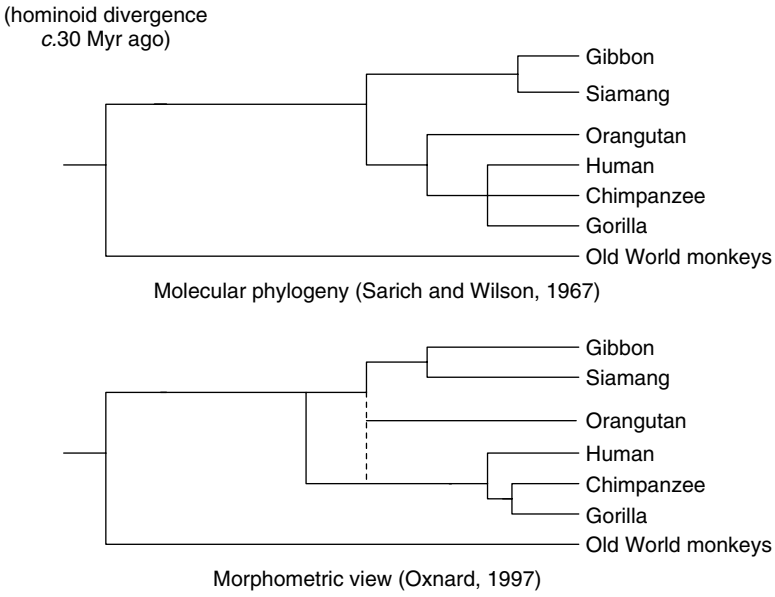

Figure 1.1. Sarich and Wilson's hominoid phylogeny (Sarich and Wilson, 1967), based on an immunological approach, is shown as a representative molecular phylogeny. Below this is the result of Oxnard's whole-organism morphological study (Oxnard, 1997). The most notable differences are that the morphological data are inconclusive as to whether or not the Asian apes form a clade (as indicated by the dashed line in the figure), and the relative timing of the human–chimpanzee–gorilla split. An approximate linear time scale spanning 30 million years can be applied to the molecular phylogeny. The morphological phylogeny is drawn so as to indicate a rough correspondence in this respect, although morphological data do not allow imposition of a time scale in the same way as do molecular data.

classified as *derived*, and may be shared within the group as a result of either homology, i.e. common ancestry, or homoplasy, i.e. similarity due to either independent mutation (*convergence* or *parallelism*) or reversion to a primitive state (*reversal*). Homoplasy acts to obscure phylogenetic relationships by giving a false appearance of shared ancestry, and so it is the identification of shared derived characters due to homology that leads to the construction of the most likely phylogeny. A group of species comprising a common ancestor and all its descendants is known as a *clade*, and is the fundamental unit of a cladistic classification.

Whatever approach is employed, morphological or molecular, there are a number of intrinsic limitations that must be recognised and, if possible,

overcome (Cronquist, 1987; Sokal, 1985). There are fundamental problems with the raw data. Morphological methods are most limited by the fact that there is no simple and reversible mapping between observable features and the underlying genetic programming, and by the fact that some characters reflect non-hereditary development, e.g. via the effects of biomechanics on bones, joints and muscles, i.e. external effects due to individual activity patterns (although these may be selected when of genetic origin). In more detail, hereditary character states may be the result of random mutations, or selected hereditary adaptations to some long-continued factor such as climate change, or be functionally adaptive (i.e. a selected hereditary character). Non-hereditary characters may also be the result of some randomness (e.g. due to disease), or be directed (e.g. in ontogenetic response to continued undernutrition), or be functionally adaptive, i.e. produced during ontogeny by interaction with the home environment of each species.

Furthermore, treating any particular character (and its associated states) as representative of some single evolutionary entity that may be meaningfully classified as primitive or derived for phylogenetic purposes, as described above, is a drastic oversimplification. Each morphological character, i.e. observable feature, is a complex of potentially quite a large number of underlying characters, and these are, of course, a mixture of various types. It is therefore not even theoretically possible to unambiguously classify the character under consideration. Further complications arise because of interdependencies between the underlying characters, meaning that, even if desired, it is difficult to quantify the contributions of the underlying characters in a way that preserves the ability to compare across characters. Oxnard (2000) discusses specific techniques for identifying parallels and convergences in morphological data, and thus avoiding confusion in phylogenetic interpretations.

For example, the character ‘cranial length’, considered primitive in apes when long and derived in humans when short, provides a useful illustration of this problem. Cranial length actually comprises several lengths, each resulting from a mix of hereditary or non-hereditary effects with varying degrees of uncertainty. Firstly, the length of the outer table of the skull at the front is probably related to load-bearing and may depend upon both heredity (thicker skulls may run in a family) and function during life (non-hereditary) (such as powerful chewing of skins). Next is the thickness of the diploe (i.e. the marrow cavity within the skull bone at the front), which may be related to blood-forming (probably hereditary), and perhaps also to biomechanics with the bone operating in a poroelastic rather than an elastic mode (probably non-hereditary). The length from front to back of the frontal air sinus is perhaps related to the physiology of the respiratory system and perhaps

also to stress-bearing; these characteristics could be either hereditary or non-hereditary. Then there is the length of the anterior fossa of the cranial cavity, which contains the frontal lobes of the brain but is also dependent upon the size of the eyeball, and next the length of the middle cranial fossa, containing the parietal lobes but also dependent on middle- and inner-ear adaptations. The length of the clivus is probably related to brain and skull growth, but also partly of somitic origin and therefore dependent on somite origin and growth. The length of the foramen magnum depends on the size of spinal cord, and thus on the input–output relationship between body and brain. The length of the occipital planum depends on the size of the posterior lobes of the brain, but maybe also on the strength of the nuchal muscles. Then there are three more measures relating to the inner table of the occipital bone, the diploe at the back and the outer table of the occipital bone.[2]

Obviously all these 'characters' could have, and should have, different cladistic designations. What, then, is to be made of the overall character of cranial length? Certainly a simplistic classification as either primitive or shared derived cannot capture anything like the complete picture.

Classification difficulties aside, the lack or incompleteness of relevant fossils, and problems in the identification of fossils, creates difficulties that must also be overcome. For example, no gorilla or chimpanzee fossils have been identified; all candidate fossils from the past 6 million years have been placed on the human line (Gee, 2001). Molecular methods are limited by the facts that DNA mutates at an unknown rate, is differentially selected based on its consequent selective advantage or disadvantage, is mixed each generation, and its transfer is restricted in various ways, e.g. owing to geographic constraints, migration and breeding patterns (Avise, 2000).

Once the data have been obtained and identified, applying the method described above also involves overcoming a number of practical complications. Identification of appropriate characters is the first problem. It is important that the characters used are independent of each other, and properly reflect evolutionary history and not individual adaptation. Once the characters have been identified, classification of their states as primitive or derived, and, if shared derived, homologous or homoplasious, is difficult and prone to error. Then there are a number of different methods for constructing the phylogeny, often producing no single best choice. The computational methods required are intrinsically hard,[3] meaning that there exists no reasonable (polynomial) time algorithm able to guarantee finding the best solution: as the number of

[2] See, for example, Oxnard (2004) for a related discussion.
[3] In mathematical terms, they belong to the family of *NP-complete* problems (Day *et al*., 1986; Graham and Foulds, 1982).

characters is increased, the search time required to locate the best phylogeny increases exponentially. Furthermore, results based on different measures are often inconsistent, and are sensitive to the ordering of characters, the order of input, the approximations employed to overcome limitations of the chosen algorithm, etc. (Felsenstein, 1982; Sokal, 1985).

Despite these problems, the general agreement regarding the evolutionary relationships between humans and apes that has emerged since the advent of molecular studies has proven quite robust.

1.2 Origin of modern humans

When fossils of archaic humans are included in the above picture, things become much less clear. Despite the general agreement on the evolutionary relationship of humans and apes, when it comes to details of the human lineage there is substantial disagreement over issues such as the rate of evolution, the number of distinct evolutionary lineages involved, the extent of interbreeding, and what migrations have occurred (Relethford, 1998), leading to serious contention in the matter of modern human origins.

The argument is usually presented as a conflict between two models (Smith and Harrold, 1997). According to the *Recent African Origin* or *Replacement* model, anatomically modern humans emerged as a new species in Africa around 200 000 years ago, and then spread throughout the Old World, replacing existing populations without significant interbreeding. Opposed to this is the *Multiregional Evolution* or *Regional Continuity* model, which views all human evolution as taking place within a single evolutionary lineage. According to this model, modern humans arose simultaneously everywhere, as a result of interregional gene flow. These two models are extreme positions on a spectrum of such models, each hypothesising various degrees of replacement and continuity.

A great deal of research involved in the attempt to distinguish between these possibilities concerns mitochondrial DNA. Mitochondria are small cellular organelles important for metabolism, and each contains a small (*c.* 16 000 bp) circular genome known as mitochondrial DNA (mtDNA). Mitochondrial DNA has two very important properties that make it extremely useful in the study of modern human origins. Firstly, it is purely maternally inherited (see Avise (2000) and Strauss (1999) for a discussion of paternal leakage); secondly, it mutates an order of magnitude faster than nuclear DNA (Seielstad *et al.*, 1998), and therefore can resolve much shorter time scales. Polymorphisms in the mtDNA data allow the construction of a phylogeny, and given an estimate

of the effective population size, i.e. the number of breeding individuals over the period of interest, and an estimate of the mutation rate, a time may be assigned to the depth of the tree, and to the migrations therein.

Cann *et al*. (1987) studied mtDNA collected from 147 individuals of Asian, Australasian, European and African ancestry. The phylogeny they constructed had two branches, one of which consisted entirely of individuals with African ancestry, leading to the conclusion that the common mtDNA ancestor of all humans, the so-called *Mitochondrial Eve*, lived in Africa. This conclusion was further supported by the fact that the African populations showed the greatest variation in their mtDNA, an indication of greatest age. To determine the time of this common ancestor, an estimate of the mutation rate of mtDNA was required. Cann *et al*. obtained this by assuming a constant rate of mutation together with a date of 5 million years ago for the human–chimpanzee common ancestor. Given the present-day divergence of human and chimpanzee mtDNA, this led to an estimate of between 140 000 and 290 000 years ago, a date strongly in agreement with the Recent African Origin model. Many criticisms of the method employed have been addressed in later work (see, for example, Vigilante *et al*. (1991)).

The paternal analogue to mtDNA is provided by those parts of the Y chromosome that are not homologous to the X chromosome (Jobling and Tyler-Smith, 1995). Recent studies of Y-chromosome polymorphisms have mostly concurred with the mtDNA picture (Dorit *et al*., 1995; Hammer, 1995; Pritchard *et al*., 1999; Underhill *et al*., 1997; Whitfield *et al*., 1995) but have also acted to bring into focus the effect on the statistical analysis of the underlying demographic assumptions employed in these studies. Fu and Li (1996) reanalysed the results of Dorit *et al*. (1995) and showed that there is such a substantial dependence on the estimate of N, the effective population size, that it can change the estimate of the time of the most recent paternal common ancestor by an order of magnitude, with the mean ranging from 92 000 years for $N = 2500$ up to 703 000 years for $N = 30\,000$. Brookfield (1997) considers several such estimates, and concludes '... the estimates depend hardly at all on the data, and almost entirely on the demographic model assumed.'

There has also been the suggestion that mtDNA results imply a severe population bottleneck and that all modern humans are descended from an extremely small and recent founder population, even a single individual. Ayala (1995) addressed and thoroughly dismissed this suggestion, claiming that the data actually imply that the effective population never dropped below 100 000 individuals (although the size of this figure is now understood to be a result of balancing selection; see Sherry *et al*. (1998)). The consensus is for a population of the order of 10 000 breeding individuals (Gagneux *et al*., 1999; Relethford, 1998; Sherry *et al*., 1998), with evidence for a relatively

recent demographic bottleneck or selective sweep in human origins. It is interesting to note that for both mtDNA and the Y chromosome, the variation within humans, when compared with other primates, is surprisingly small, also implying a relatively recent divergence.

Similar work has been done using genes on autosomes, although the situation is far more complicated because of the two potential paths of inheritance each generation, and the corresponding greater depth of the resulting phylogenies. Harding *et al*. (1997) studied 326 sequences of the beta-globin gene, and found African common ancestry, dated at approximately 800 000 years ago, and no evidence of the effective population dropping below 10 000 individuals at any time. More significantly, they found a depth of greater than 200 000 years in their Asian sample, implying that the ancestral population was already widely dispersed at that time. A similar challenge to the Recent African Origin model comes from the analysis of the mtDNA of the *Mungo Man* (Adcock *et al*., 2001), an anatomically modern human found at Lake Mungo, Australia, and dated at about 60 000 years ago. It was found that despite being anatomically modern, his mtDNA lineage diverged before the most recent common ancestor of living human mtDNA. Relethford (1998) demonstrates how this entire class of results can be interpreted from a population perspective rather than from a phylogenetic perspective, and thus be shown to be consistent with both a recent African origin and multiregional evolution.

It must be remembered that a species tree is actually a combination of several individual gene trees, and the overall picture may only be recoverable through the study of several of these individual genes (Moore, 1995). The three species shown in Figure 1.2 contain a gene whose form in species C is older than the form in species A and B (the B–C species ancestor being polymorphic). Sampling this particular gene would incorrectly imply a closer relationship between species A and B than between B and C. (Analogously, in a morphological study, many independent morphological characters may be needed for accurate resolution of a species tree.)

There are problems not only with sampling effects and the underlying demographic assumptions as discussed above, but also with the assumptions regarding the other important input: the mutation rate.

As is apparent from the above discussion of the work by Cann *et al*. (1987), molecular methods rely on knowledge of the mutation rate of DNA across time and between species. The *molecular clock hypothesis* is a consequence of the neutral theory of evolution (Kimura, 1968) and implies an approximately constant rate of mutation, so long as the DNA sequence retains its original function. If this is the case, then the degree of difference between sequences being compared is simply proportional to the time since

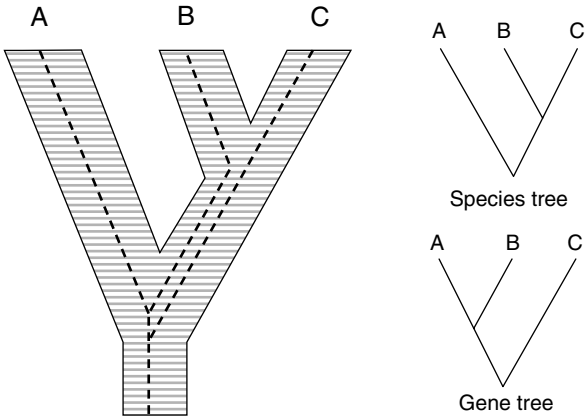

Figure 1.2. A phylogeny of three species, with the path of a particular gene shown by a dashed line. The simplified species tree and conflicting gene tree for these three sample species are shown on the right.

the sequences diverged. By incorporating fossil evidence, the *clock* can be calibrated, and thus divergence times can be attached to a molecular phylogeny.

In fact, particular DNA sequences and proteins can mutate at vastly different rates at different times and in different lineages, and although there may be some local validity of the molecular clock hypothesis, in general there is global failure (Avise, 2000; Gibbons, 1998; Ruvolo, 1996; Strauss, 1999; Wills, 1995). The fast-mutating microsatellite loci, i.e. short repetitive sections of DNA that lie between genes, have been used to construct an alternative method for timing lineages that does not rely on external calibration of the rate of molecular evolution (Goldstein *et al.*, 1995). However, because of mutational saturation, nuclear microsatellites are only useful for timing relatively recent events. In particular, the deepest split in the human phylogeny can be recovered with such a method, but saturation will occur in less time than the five million years or more back to the human–chimpanzee common ancestor (Jorde *et al.*, 1998).

This situation also affects substantially the common ancestor calculations described above. For example, Wills (1995) includes a variable mutation rate across mtDNA sites and obtains a range of 436 000 to 800 000 years ago for the mitochondrial common ancestor, depending on the date used for the human–chimpanzee common ancestor.

In general, the molecular data seem to support the replacement hypothesis, but when all the aforementioned caveats are considered, it remains far from