CHAPTER TWO

Human Evolutionary Genetics

The basic principles of evolution provide the framework within which the origin and development of *Homo sapiens* can be understood from a biological perspective. The study of molecular evolution has primarily been concerned with the origin of species and especially with the origin of *Homo sapiens*, the adaptation of different species to their environment and the trends within groups of related species through the study of changes over time (Marshall *et al.*, 2011). Understanding the evolutionary changes within human populations is the key to understanding the genetic makeup of a group of individuals, which includes important aspects, such as improved understanding of disease aetiology.

2.1 THE FUNDAMENTALS OF EVOLUTIONARY THEORY

The first theory of evolution was established by Charles Darwin and Alfred Wallace (Darwin and Wallace, 1858; Wallace, 1858) who hypothesised that the fundamental changes that were observed between organisms could be ascribed to positive selection that enabled the organisms to adapt to their environmental challenges and thus survive. Darwinism stipulated that in order for an organism to survive, it had to reproduce, thus carrying over characteristics necessary for that survival, which introduced variation between individual organisms and created competition within a population for survival. Darwin was the first to introduce the concept that evolutionary processes shaped the genetic variation that contributed to the fitness of a population of organisms, which was, according to his theories based on natural selection (Marshall *et al.*, 2011). This was followed by a model of inheritance devised by Mendel, which is widely regarded as the beginning of the field of human genetics and has developed over time into a broad science that covers many different fields of applications. One of these is the application of genetic science to the theories of evolution. Through Mendelism, the concept of genes as units of inheritance was introduced and identified as the carriers of the changes of evolution. The mechanisms that introduce hereditary variation are therefore central to the study of evolution and refer to the study of variation in populations rather than in individuals (Pearson, 1999). The pioneering work of Fisher, Wright and Haldane led to the development of population genetics, which established the importance of the study of the

forces that shape the genetic variation within human populations (Fisher, 1930; Haldane, 1932; Wright, 1986).

As the field of molecular genetics developed, the theories of Darwin were evaluated and investigated in terms of the principles of genetics, which led to the development of the advanced evolutionary theory of Modern Synthesis as developed by such scientists as Dobzhansky (1937), Huxley (1942), Mayr (1944) and Simpson (1944). This theory is based on the assumption that genetic variation is random and unidirectional and the principal force behind evolution. It further emphasises the importance of natural selection as a driving force of evolution through the fixation of advantageous sequence variants and the elimination of deleterious sequence variants through positive and negative selection respectively. The theory of Modern Synthesis further incorporates the random fixation or loss of sequence variants through the process of genetic drift, which is primarily dependent on the effective size of populations (Wright, 1986). Therefore, the evolutionary theories developed during the period between 1940 and 1990 went from acknowledging that variation occurred within populations and species because of the process of natural selection to an extension of these concepts to include the principles of continued genetic variation, gene flow and genetic adaptation to environmental challenges as well. Subsequently, emphasis was placed on geographical genetic variation, the role of population isolation, genetic bottlenecks, population dispersal, replacements and extinctions.

Wright's concept of genetic drift as an evolutionary force was further developed into a neutral theory of molecular evolution by Kimura (1971), who hypothesised that the majority of mutations that were fixed in a population were selectively neutral and were fixed through the random process of genetic drift rather than through selection. It was concluded that the probability that two samples taken from a population will differ at a specific position is determined by the mutation rate and the size of the effective population (Kimura, 1971; Kimura, 1991). With the development of this theory, the concept that sequences evolve in a clock-like manner came into play and it was proposed that evolution is governed by the stabilising purifying selection that eliminates deleterious mutations as they occur through a constant mutation rate. These are then fixed or lost through the process of genetic drift that is high in small populations and low in large populations (Kimura, 1991). The neutral theory has subsequently been refined to a nearly neutral theory to accommodate the fixation of deleterious mutations through genetic drift in small populations (Kimura, 1991).

Kimura's neutral theory of evolution is thus drastically different from the positive selection model that was proposed by Darwin.

2.2 HUMAN EVOLUTIONARY GENETICS

Genetic diversity plays an important role in understanding the history of our species. The vast amount of DNA diversity data that have accumulated over the past decade has contributed much to the understanding of human medicine and developmental biology and emphasise the importance of gaining knowledge of human diversity and by implication of human evolution (Cavalli-Sforza and Feldman, 2003).

Genetic diversity was first demonstrated in the *ABO* gene with the identification of the different blood groups in humans and was described by Hirschfeld in 1919 (Hirschfeld and Hirschfeld, 1919). The initial study of diversity among humans consisted mainly of the study of diversity of serological markers because of the limited methodologies that existed at the time. Electrophoretic techniques followed in the 1950's that broadened the scope of serological diversity studies. It was only in the 1980's that genetic diversity became the focus of studies through the use of restriction enzyme sites. The development of polymerase chain reaction (PCR) and eventually automated sequencing in the 1990's has provided the field with invaluable information and has led to the full exploitation of information on genetic variation. This has allowed the investigation of specific evolutionary events, such as the effects of selective forces on genomic regions, and has provided information with the necessary statistical power to uncover smaller complexities of evolutionary processes (Koonin, 2009). DNA diversity data have provided clarity on previously unanswered questions related to human migration and relationships of currently diverse human populations (Cavalli-Sforza and Feldman, 2003).

2.2.1 The extent and source of genetic variation

The genetic diversity of a population is determined by a complex interplay between the mutations that occur in the genomes of the individuals of that population and the evolutionary forces that shape the genetic diversity. These mutations are transferred from generation to generation by germ line inheritance and the process of segregation, and are either fixed or lost through evolutionary processes such as natural selection and genetic drift, depending on the effect of the mutation on the viability of the offspring. Sequence variation of a population is therefore determined by population parameters that represent

the values that are constant over time, and population dynamics that represent variables that change over time (Luikart *et al.*, 2003).

2.2.1.1 Mutation

The genomes of all species have the ability to introduce DNA sequence variation by mutating nucleotides within the existing DNA sequence patterns. These mutations, which are classified as transitions or transversions, are based on the type of nucleotide change that took place, usually caused by an inaccurate replication of DNA or in lesser instances, caused by environmental factors such as exposure to radiation (Lynch, 2008). Defence systems exist to protect organisms from the effects of the occurrence of mildly deleterious mutations (Eyre-Walker and Keightley, 2007). These include the inherent strong tendency of the replication polymerase enzymes to incorporate complementary bases to those in the template strands during DNA replication and verify the accuracy of the process by a proofreading mechanism that would remove the base misincorporations, revise the accuracy of the base incorporations by a postreplicative mismatch-repair system and lastly use the forces of natural selection as final arbiter to remove deleterious mutations from the DNA genomes on a population level (Lynch, 2008). Mutational events that lead to an insertion or deletion of nucleotides in DNA sequences are also possible but rare, since these types of mutations usually have a seriously deleterious effect on the genomes owing to frameshifts in coding regions of the genomes. Synonymous mutations that do not lead to a change in the expressed amino acid usually occur in the third codon position, as opposed to nonsynonymous mutations that often occur in first and second codon positions (Eyre-Walker and Keightley, 2007). Germ line mutations are carried over from one generation to the next and are therefore the basis of evolutionary change, as opposed to mutations that have occurred in the somatic cells, which are not carried over to subsequent generations (Pages and Holmes, 1998).

The process of nucleotide substitution is therefore ultimately responsible for the long-term variation in the nucleotide composition of the genomes of most species. This happens when mutations are fixed in the genetic composition of a population and it is expected that the rate at which the mutations occur and the rate at which they are fixed should be equal under conditions of the neutral theory of evolution as discussed in the previous section (Kimura, 1968). When these assumptions are rejected, however, the substitution and mutation rates will differ and mutations would not necessarily be fixed in a population. The

mutations are maintained as transient polymorphisms in the population during the period between the occurrence and the fixation of the mutation (Kimura, 1971).

The observation that genetic variation increases in a linear fashion over time has led to the development of the concept of a molecular clock, which states that mutation and therefore evolution happen at a constant pace over time (Hedges and Kumar, 2003). Within the framework of the neutral theory, where it is assumed that a population is constant in size, that mating is random and that mutations are neutral, the mutation rates for individual species could be determined and this was the beginning of much research into the large differences in the mutation rate between species (Kumar and Subramanian, 2002). The use of the molecular-clock principle to estimate divergence times between species followed and has led to the estimated TMRCA in humans and many other organisms (Kumar and Subramanian, 2002). The extensive work on the mutation rates between species and within the genomes of singular species has provided evidence that the mutation rates differ between species and also within the genomes of individuals of one species (Sanderson and Shaffer, 2002). These findings provided evidence that different genes evolved at different rates because of natural selection and gene function and therefore that the molecular-clock principle should be applied by using relative rate tests and methods that can estimate time within the restrictions of sequence variability (Hedges, 1992; Hedges and Kumar, 2003).

The rate of mutation varies across the human genome, with the Y chromosomes evolving faster than the X chromosomes, most likely because of a higher mutation rate in males (Li and Makova, 2002). It also varies between the human nuclear genome and the mitochondrial genome. The latter displays much higher mutation rates than the genomic DNA, making this uniparental haploid marker a carrier of large amounts of sequence variation (Stoneking and Soodyall, 1996). The mutation rate also varies between humans and other organisms, animals and plants and is generally thought to be connected to a complex interplay of the rate of DNA replication, the size of the genomes and the effective population sizes within each of these groups (Lynch, 2010).

The autosomes and mitochondrial genomes of humans further display variable rates of mutation in different regions of the genomes. The reasons for this phenomenon in the autosome are unclear and in the mitochondrial genome it is connected to the regions of coding and non-coding DNA (Hodgkinson *et al.*, 2009). This variability can be observed in its most dramatic form at the nucleotide level where mutation rates can differ between

nucleotide sites. The high incidence of the methylation of cytosine-phosphate-guanine (CpG) dinucleotides in mammals has been cited (Bird, 1980) as a likely reason for this because it causes unstable methyl-cytosine bonds and causes a directional substitution of cytosine to thymine (C-T) and guanine to adenine (G-A). The presence of regions of hypermutability in the genome has been described as a mechanism by which the organism can adapt to challenging environmental changes and therefore ensure its survival (Page and Holmes, 1998). The hypermutability at certain mutational hotspots has led to an excess of homoplasies observed in the mitochondrial genome and coincides with a similar phenomenon in the nuclear genome, which causes cryptic regions of variability that have also been connected to hypermutability within those regions (Stoneking and Soodyall, 1996; Hodgkinson *et al.*, 2009). A strand slippage model has been described as the most likely reason for this occurrence in the mitochondrial genome resulting in base substitutions through the mechanism of a transient misalignment dislocation system (Kunkel, 1985). This phenomenon is not understood in the nuclear genomes of humans as yet (Hodgkinson *et al.*, 2009).

Furthermore, much higher rates of mutation than of substitutions have been reported. This is most probably due to the effects of purifying selection that removes deleterious transient polymorphisms from the population and may mean that most of the mutations that occurred in human genomes are deleterious and would require large effective populations to persist (Woodhams, 2006). As with the previous aspect of hyper-mutability, the exact reasons for this phenomenon are not currently known and identification of the cause would depend on large genome scale datasets for further investigation (Hodgkinson *et al.*, 2009).

2.2.1.2 Inheritance of genetic variation

Genetic variation is not only caused by the occurrence of mutations, but also by the way in which the sequence variants are carried over from one generation to another. In diploid organisms, the process of recombination is an additional source of genetic variation that is introduced through the shuffling of mutations and substitutions among chromosomes during gametogenesis. Recombination therefore does not create new mutations and thus does not add to the total sequence variation but enhances it by repositioning the sequence variants between the combinations of alleles. The purpose of recombination is primarily to assist the organism to adapt to changing environments and thus increase its fitness (Pages and Holmes, 1998).

The degree of linkage disequilibrium (LD) contributes to the complete description of genetic variation in the genome because it provides an estimate of the degree of correlation between the sequence variants at different positions. The stronger the LD, the stronger the possibility that two sequence variants at different positions would be observed together. The pattern of LD is influenced by the extent of selection and the way in which both sequence variants at different positions respond to the selective forces as well as to the population scale forces of genetic drift, migration and mating. The concept of LD is therefore not always straightforward and the initial theory that the higher the degree of recombination, the lower the degree of LD between two sequence variants has been called into question. It seems more likely that LD occurs in isolated spots across the genome where it indicates low recombination in regions that display high LD and the other way round (Cavalli-Sforza and Feldman, 2003).

Inheritance of the mitochondrial genome is uniparental and although this organelle contains the machinery for cell recombination, evidence suggests that recombination is absent in human and most animal mtDNA but not absent in fungal and plant mtDNA. The absence of recombination offers great advantages for the phylogenetic reconstruction of genetic variation because of the absence of the shuffling of sequence variants. The disadvantage of the lack of recombination is that the whole mitochondrial genome is in full LD and therefore influences on the mitochondrial genome will affect the whole genome and not only isolated spots that are linked (Ballard and Whitlock, 2004).

2.2.2 Genetic drift

Neutral mutations that arise in populations would fluctuate randomly from generation to generation and eventually reach an equilibrated state, at which point the mutation would either be fixed in a population or lost from the population (Kimura, 1969). This transition of a mutation to a substitution is therefore dependent on being chosen through the process of random sampling to be carried from generation to generation until it becomes fixed as a substitution and therefore contributes to changing allele frequencies. This process of genetic drift is one of the most powerful forces of evolution, especially within the framework of the neutral theory of evolution, which stipulates that most mutations are neutral and therefore not exposed to selective pressure (King and Jukes, 1969). The longer genetic drift is present in populations, the more alterations would eventually be removed by this process of stochastic sampling and therefore reduce the genetic variation in a population.

2.2.2.1 Effective population size

Under the probabilities of random sampling, the chances of the removal of mutations would be high in a small population and low in large populations. Population size therefore plays an important role in the effects of genetic drift (King and Jukes, 1969). The concept of effective population size is used to indicate the number of individuals of a specific population that can successfully participate in reproduction and therefore in passing the mutations from generation to generation. The effective population size (N_e) would be smaller than the census population size because of the natural phenomenon that not all individuals within a population would be able to reproduce successfully (Ballard and Whitlock, 2004).

When a population experiences a sudden population reduction because of natural disasters or disease, or when population size is reduced because of the migration of a certain proportion of the population away from the initial population to form a new population, usually in a new location, it has a large impact on the sequence diversity within the populations of reduced size (Rogers, 1997). Population bottlenecks cause a drastic loss of sequence variation because of the physical reduction of sequence variants due to the loss in the numbers of living individuals from the population, which would lead to an increase in inbreeding and eventually in the effects of genetic drift. In new populations established by the founder effect, the number of individuals that would be separated and isolated from the initial populations would be low and therefore contain only a proportion of the sequence variation that was present in the initial population. The same effects of inbreeding and genetic drift will occur and this will change the genetic structure of the population totally from the genetic structure of the initial population from which it originated (Jorde *et al.*, 2000).

2.2.2.2 Population subdivision

Genetic evidence has demonstrated that the genetic differences between populations are relatively small and that the largest component of the genetic variation lies within populations (Jorde *et al.*, 2001). Analyses of polymorphisms in 14 populations that represented all continents demonstrated that 85% of the total variance was observed within the populations. The studies of evolutionary history are based on the rest of the genetic variation between populations, which consisted of 5-15% of the total variation

(Cavalli–Sforza and Feldman, 2003). The importance of knowing the genetic variation between and within populations lies in the assessment of the impact of genetic diversity on physical characteristics, susceptibility to disease and treatment options between populations (Bamshad *et al.*, 2003).

The concept of genetic drift refers to the random sampling of sequence variants from one generation to the next. This could be interpreted as the random mating between individuals of a population through which mutations would be fixed or lost. Random mating, however, is not always a realistic assumption for a natural living population, since mating is seldom random because of population substructure, which would prevent or deter individuals from mating across those borders and creating partially isolated subpopulations. This could be caused by physical separation between populations, such as distance or natural entities such as rivers or mountains, or by socio-economic or socio-cultural reasons. As in the case of small effective population sizes, the frequency of inbreeding will rise and the number of effectively breeding individuals would be less than the total population. This would lead to high genetic drift and low genetic variation, which means an increased likelihood that sequence variants that share a common ancestor would be brought together (Page and Holmes, 1998).

2.2.2.3 Migration and gene flow

Migration is an important factor that can affect the genomic variation within and between populations and should therefore be included when inferring evolutionary history from genetic variation data. When the borders of population substructure are crossed and individuals move between islands of subpopulations or major populations, mating becomes more random and mutations that occurred within the genetic structures of different populations flow between the two populations or subpopulations. Gene flow leads to an increase in genetic variation through the introduction of new sequence variants to other populations that did not harbour that type of sequence variants before and thereby increases the genetic variation within populations (Page and Holmes, 1998). Gene flow and genetic drift exhibit opposing effects on the genetic variation of a population and the genetic diversity between two populations is therefore a balance between the forces of genetic drift and gene flow. Only one immigrant per generation is needed to keep mutations from fixating in a population (Cavalli–Sforza and Feldman, 2003).

A different scenario develops when a whole population migrates to a new location and joins another population. The founder population will display large genetic differences within the initial population and also within the new population in which it has settled. This scenario will increase the effects of genetic drift and therefore decrease the overall genetic variation in the newly established combined population. The opposite is true when only a few individuals migrate to neighbouring populations and thereby increase the genetic variation (Cavalli–Sforza and Feldman, 2003). The presence of certain specific sequence variants in different populations that are located in different regions, for instance, could be used to trace the movements of the individuals of those populations to other regions and can assist in determining patterns of migration (Ballard and Whitlock, 2004).

2.2.3 Natural selection

Evolutionary literature lends great importance to the role of selection in shaping genetic variation within and between populations because it could assist in the detection of genomic regions that are of functional importance and most importantly, it provides evidence of regions of the genome that are involved with the adaptation of an organism to new environments and environmental challenges (Kimura, 1991). Furthermore, insight into the processes of natural selection and whether positive selection is indeed the principal shaping agent of genetic variability within and between species has been debated extensively and this debate continues (Nielsen, 2005).

Mutations that do not affect the fitness of the organism in which they have occurred, are regarded as neutral. Opposite to neutrality lie the mutations that affect genomes and protein functionality either negatively or positively. Deleterious mutations are either strongly deleterious when they severely compromise the fitness of the organism or mildly or weakly deleterious when the fitness of the organism is only moderately or slightly affected. Purifying selection removes the deleterious mutations as opposed to adaptive or positive selection, which enables the retention of positive mutations to allow the organism to adapt to new environmental challenges. Directional selection is predominant in an organism when the different genotypes have unequal degrees of fitness and selection becomes balancing when it conserves a heterozygotic state to ensure optimal fitness. Balancing selection is not possible in haploid genomes, because the mutations are inherited uniparentally, and is thus rather viewed as directional, preferring one or the other of the mutations present at a nucleotide site (Nielsen, 2005). Later concepts, such as the observed phenomenon that variability is sometimes decreased in sites linked to a selected

mutation, have been identified and described through the investigation of larger genomic datasets (Kaplan *et al.*, 1989). The patterns of these selective sweeps are complicated and need even larger comparative genomic and single nucleotide polymorphism (SNP) datasets to understand the exact underlying processes of selection (Nielsen, 2005).

During the early period of Darwinism and Neo-Darwinism, initial approaches tried to explain the forces that orchestrated genetic variation. Scientists were of the opinion that natural selection was the main shaping agent of the sequence variation that was observed in the genomes of nearly all species (Gillespie and Langley, 1974). In contrast to the Darwinism theory that attributed genetic variation to the purifying or adaptive forces of selection, Kimura (1971) developed a different evolutionary theory that regarded most mutations as being neutral and dismissed natural selection as the principal mechanism responsible for shaping the genetic variation within populations. Kimura (1971) argued that synonymous mutations were the least damaging to an organism and therefore possibly selectively neutral and that even nonsynonymous mutational changes to amino acids that did not affect the active sites of the protein or did not alter the tertiary structure of the proteins, would probably not have a strong negative influence on the functionality of the altered proteins. Based on the severely deleterious effects of the rare chain terminating mutations, frame-shift mutations and mutations that accompany large deletions, Kimura proposed that most mutations are selectively neutral at the majority of sites in the genome (Kimura, 1971). This theory is known as the neutral theory of evolution and predicts that new mutations in a population may increase in number although they do not contribute to the fitness of the organism and that these frequencies are driven by evolutionary processes such as genetic drift (Kimura, 1971). Later adjustments to the neutral theory acknowledged the role of purifying selection in the removal of the minority of mutations that are severely deleterious (Charlesworth *et al.*, 1993).

Natural selection modifies the genetic variability within and between species on different levels. Selective sweeps are reported to cause drastically reduced levels of genetic variability within species and not necessarily between species, whereas negative selection has been reported to reduce genetic variability between species rather than within species. Overall, selection has been reported to often increase the degree of genetic differentiation between populations and therefore contribute to the genetic subdivision of populations (Charlesworth *et al.*, 1995). Different types of selective processes can be identified from observing the frequency spectrum of the allele frequencies of mutations in a sample of sequences. Purifying selection will decrease the number of low-frequency recent or private

mutations, as it removes the deleterious mutations from a population as opposed to positive selection, which will cause an increase in the selected adaptive mutations that will eventually become fixed in the population. This is especially evident from a decrease in nonsynonymous mutations under purifying selection and an increase in nonsynonymous mutations under positive selection when compared to the number of synonymous mutations in a sample of sequences (McDonald and Kreitman, 1991; Smith, 1994). LD and haplotype structure are regarded as other genetic signals of selection in the genome. It has been observed that the level of LD will increase in regions of the genome that are under selection and that the selective sweeps cause distinct haplotype patterns that can be identified (Hudson *et al.*, 1994; Sabeti *et al.*, 2002).

The development of technology and the increased availability of sequence data have improved the understanding of the complexity of the interplay between natural selection and other evolutionary forces. Selection has proven to be pervasive because of the difficulty in distinguishing between the genetic signals caused by selection, such as the increase in the frequency of certain sequence variants, and the genetic signals caused by other evolutionary forces such as the effects of population expansions that would, for example, also produce an increase in certain sequence variants. Another example would be the modern theories about selective sweeps that hypothesise that selection is not only dependent on the degree to which the mutation affects the fitness of the organism, but is also dependent on the loci linked to the selected mutation and that genetic differentiation between species is created by these sets of neutral mutations that are linked with the adaptive mutations and sweep through populations (Gillespie and Langley, 1974). The main questions with regard to the role of selection in evolution would be to resolve the exact roles and complexities of natural selection and genetic drift and to determine how these two major evolutionary forces cooperate to create a genetic framework from which evolutionists can interpret the evolutionary history of the species of the world.

2.2.4 Genetic markers used to study genetic variation

The study of the differences between humans and human populations started with the quantified analyses of protein polymorphisms (Cavalli-Sforza and Feldman, 2003). The study of genetic diversity became highly resolved with the introduction of technologies such as PCR and automated nucleotide sequencing analyses. Techniques such as denaturing high-pressure liquid chromatography, single-strand conformational polymorphism analysis and automated direct sequencing, such as reduced-representation

shotgun sequencing (RRSS) in combination with next generation sequencing technologies, are used to determine the nucleotide variability within population samples (Mir and Southern, 2000).

Autosomes and X chromosomes are good markers to use in the study of ancient evolutionary histories that stretch back over millions of years because they evolve more slowly than the Y chromosome markers and mitochondrial genomes (Nachman and Crowell, 2000). These haplotypes, which consist of short regions of a chromosome that contain a specific combination of sequence variants, are analysed by using SNPs. SNPs are used to investigate genetic diversity, recombination and LD. Other markers that are also used to investigate the evolutionary history of populations include insertions and deletions, which can range in size from small to large regions that are inserted or deleted. These indels are often used in studies of gene mapping to distinguish chromosomal lineages because they occur so rarely and seldom undergo reverse mutational events (Weber *et al.*, 2002).

Retroposable elements such as short interspersed nuclear elements (SINEs) and long interspersed nuclear elements are valuable genetic markers because their ancestral states are reported and they are abundant in the human genome (Batzer and Deininger, 2002). *Alu* inserts are popular SINEs in the human genome for use in evolutionary studies because they are abundant and can therefore easily be detected. In addition, the number of repeats within *Alu* inserts can be used to detect ancestral lineages and detect migrations and population differentiation events of a population (Jorde *et al.*, 2000).

The more rapidly evolving genetic markers consist of microsatellites, minisatellites and haploid markers such as the Y chromosome and mitochondrial genomes. The higher mutation rates within these genetic markers make them carry high loads of sequence variation, which is a great advantage in the study of evolutionary history (Jeffreys and Neumann, 1997). Sex-based aspects of evolutionary histories of populations can be investigated by using maternally and paternally inherited haploid markers such as the mitochondrial genomes and the Y chromosomes. Both these markers portray the ancestral lineages of a single sex only and thereby provide valuable information with regard to the similarities or differences between the female and male behaviours of early populations (Jorde *et al*., 2000).

The mitochondrial genome is especially valuable in the study of evolution because of its high mutation rate in comparison to the autosomal markers and the fact that it is inherited uniparentally without recombination, thereby facilitating the tracking of ancestral lineages (Wallace *et al.*, 1999). Coding and non-coding regions of the mitochondrial genome are investigated by restriction fragment length polymorphism (RFLP) or by whole genome nucleotide sequencing (Horai *et al.*, 1995; Wallace *et al.*, 1999). Although it is commonly believed that Y chromosomes are also inherited uniparentally, they display recombination in certain regions of the chromosome. It also displays a much lower level of genetic variation when compared to the autosomal chromosomes and therefore has its greatest value in the genetic patterns of paternal inheritance that it provides (Horai *et al.*, 1995). Both haploid markers display regions that are associated with disease and therefore are submitted to the effects of selection. The effects of selective sweeps are especially high in these markers because of the absence of recombination, which effectively links the nucleotide sites to form a single locus that will display drastically lower levels of neutral variation under conditions of selective sweep. This in turn might affect the haplotype phylogenies and time estimates and therefore these markers are regularly investigated for genetic signs of selection in order to interpret the patterns of genetic variation in the context of demographic forces to which they were exposed (Charlesworth *et al.*, 1995; Bamshad *et al.*, 2003).

2.3 EVOLUTIONARY HISTORY OF MODERN HUMANS IN AFRICA

Although the African continent has not been studied to its full extent from a molecular anthropological point of view, large studies have investigated the genetic diversity of many of the current African populations with the purpose to obtain greater insight into the evolutionary history of early modern humans and subsequent developments (Salas *et al.*, 2002; Mishmar *et al.*, 2003; Behar *et al.*, 2008; Tishkoff *et al.,* 2009). This has provided a broad evolutionary history that starts with the modern humans that originated in Africa about 200 thousand years ago (kya) (Vigilant *et al*., 1991) and that have inhabited the African continent since then continuously. The African populations have maintained relatively large population sizes (Campbell and Tishkoff, 2010) and therefore display an invaluable amount of genetic diversity, cultural heritage and linguistic variability, since they live under a broad range of environmental conditions. The different environmental conditions have resulted in the development of many different lifestyles to adjust to the climate and vegetation or lack thereof. The most common subsistence strategies are hunting-gathering, agriculture and pastoralism. The dispersal of modern humans from a

single region of origin to populate the rest of the continent meant that major migration events took place, which had a large impact on the genetic diversity within and between populations as they separated, admixed, expanded and experienced population bottlenecks through the ages (Rosa and Brehm, 2011).

2.3.1 Origin of modern humans in Africa

The study of evolution is approached by the integration of evidence from fossils, archaeological records and genetic diversity data. Recent human evolution has been determined by distinguishing between archaic humans and anatomically modern humans (AMH) through interpretation of evidence of the transitional phase between the two forms (Relethford and Harding, 2001). It is estimated that the early archaic *Homo erectus* dispersed from Africa into Europe and Asia about 0.8 to 1.8 million years ago (Wolpoff *et al*., 2000). Genetic mtDNA data from ancient fossils of *H. s. neanderthaliensis (*Neanderthal) have suggested a genetic divergence from *Homo sapiens* at about 600 kya (Krings *et al.*, 1997), with fossil evidence further suggesting an effective separation between the Neanderthals and modern humans at about 300 kya and further divergence among AMH at less than 200 kya (Stringer, 2002). Evidence from archaeological remains supported the fossil evidence that modern humans originated about 195-150 kya (Bar-Yosef, 1987; White *et al.*, 2003), with evidence of human behaviour dating back to about 70-40 kya during the Late Stone Age (Salas *et al.,* 2002).

Several models have been proposed for the origin of modern humans. The Recent African Origin (RAO) hypothesis, also known as the Out of Africa model, proposed that the modern humans had their origin in eastern Africa and subsequently spread out of Africa and replaced the more ancient *Homo* species that resided in other parts of the world without any admixture (Stringer, 2002). The Weak Garden of Eden model was an updated version of the RAO model and postulated that the human populations that migrated out of Africa were small and subdivided for a long period before population expansion began around 50 kya (Harpending *et al.*, 1993). The RAO model therefore suggested that all human lineages stemmed from a single ancestor, which originated in Africa, as was suggested and supported by the genetic data.

A second model, which was referred to as the Multiregion (MR) model, was developed by Weidenreich (1946) and further explored by Wolpoff *et al*. (2000). It suggested that modern-day humans originated and evolved on different continents in a parallel manner

into modern humans (Stringer, 2002). The MR model further proposed that Europe was populated by archaic *Homo* species that evolved from the early *H. s. neanderthaliensis (*Neanderthal) and *Homo erectus* populations and was concerned with the extent of gene flow and admixture between these early *Homo* species.

Both these models were, however, loosely defined and many different submodels were developed over time with regard to the exact implications of the geographical regions of origin, genetic structuring of populations and continuity of gene flow or lack thereof (Goldstein and Chikhi, 2002). These assimilation models suggested that gene flow took place between the AMH and the archaic *Homo erectus* and Neanderthal species and that the evolution of AMH populations was based on a gradual integration of features from archaic *Homo* species with more modern characteristics from the early African populations (Stringer and Andrews, 1988).

Genetic evidence is in favour of the RAO model and even to a certain extent of the assimilation models because of evidence that the Neanderthals and the AMH diverged at about 300 kya (Stringer, 2002). This has, however, not been confirmed because of the lack of large sets of genomic data that would be needed to confirm such an assimilation between the Neanderthals and AMH (Nordborg, 1998). Genetic and other evidence also does not often support the MR theory and it is generally not accepted as true by most evolutionists (Ingman *et al.*, 2000; Gonder *et al.*, 2007; Behar *et al.*, 2008). The main criticism against the MR theory is that the current genetic diversity data cannot be reconciled with the admixture of archaic Old World *Homo* populations and modern *Homo* populations and does not reflect the genetic differentiation that would be expected if such admixture took place (Harpending and Rogers, 2000). Studies done on the mitochondrial haplogroups of Caucasian individuals resulted in the MR theory being regarded as even more improbable, as the haplogroup divergence times do not correspond to a population that evolved from the *H. s. neanderthaliensis*, who colonised Europe more than 700 kya (Torroni *et al.,* 2001; Torroni *et al.,* 2006). The MR model is further challenged by recent discoveries of evidence that the Neanderthals were the only near modern human species for which evidence could only be found in Europe and West Asia (Krings *et al.*, 1997). It is estimated that the Neanderthals disappeared about 40 to 30 kya, which is the same time that modern humans appeared in those regions and that Europe was populated by modern humans migrating from the Middle East to Europe around 80 kya. The Neanderthals were presumably living in Europe at the same time that the modern humans migrated there and were most probably replaced by the modern humans (Torroni *et al.,* 2001; Torroni *et al.,*

2006). Further evidence against the MR model is that the genetic variation between populations today is so small that it could easily have accumulated in the last 100,000 years, that no anthropological continuity between fossil skulls has been found to substantiate parallel evolution and that non-African LD is much higher than the LD of African populations, indicating that the non-African group is a sub-group of the African genetic group (Cavalli-Sforza and Feldman, 2003).

Collective genetic data from uniparentally inherited mitochondrial genomes and Y chromosomes, in conjunction with autosomal genetic data, demonstrated that African DNA samples displayed the highest level of genetic diversity relative to non-African samples for all humans (Kivisild *et al.*, 2004; Atkinson *et al.*, 2009). In addition, mtDNA and Y chromosome phylogenetic trees have positioned the African samples at the deepest branches in the human phylogenetic tree with non-African samples being derived from these lineages (Gonder *et al.*, 2007; Tishkoff *et al*., 2007; Behar *et al.*, 2008; Henn *et al.*, 2008). Furthermore, mitochondrial genomes traced back to the existence of a single maternal ancestor between 200 to 160 kya in a south-eastern or eastern cradle of Africa (Ingman *et al.*, 2000; Mishmar *et al.*, 2003; Torroni *et al.*, 2006; Gonder *et al.*, 2007; Behar *et al.*, 2008), supporting the theory of a single point of origin that lies in eastern Africa.

2.3.2 The distribution of genetic diversity

High levels of genetic diversity within a population can be interpreted as evidence that the population under investigation has been in existence for a long period of time and is regularly used as an indicator of an ancestral population. The sequence variation of African populations has therefore been investigated and compared with that of non-African populations. Studies of protein polymorphisms (Cavalli-Sforza and Feldman, 2003), mtDNA sequences (Chen *et al*., 2000; Ingman *et al*., 2000), X chromosomes (Harris and Hey, 1999), Y chromosomes (Nachman and Crowell, 2000), autosomal microsatellites, minisatellites and *Alu* elements (Jorde *et al.*, 2000) and autosomal haplotypes (Tishkoff *et al.*, 1996) have all provided evidence that the African populations displayed higher genetic diversity levels than non-African populations and that African individuals were positioned at the basal branches of the human phylogenetic tree, indicating that they form the ancestral population of the current modern human populations.

MtDNA studies have determined that the African populations displayed a higher number of population-specific haplotypes than any other of the world's populations (Wallace *et al.*,

1999; Chen *et al.*, 1995a; Ingman *et al.*, 2000; Salas *et al.*, 2002). It was further determined that the non-African populations displayed a subset of the haplotypes observed in the African populations, suggesting that the non-African populations originated from an African population that migrated out of Africa and underwent a bottleneck whereby the genetic diversity was severely limited to a subset of African lineages (Tishkoff and Williams, 2002). The findings of mtDNA studies were supported by similar studies performed on Y chromosomes (Hammer *et al.*, 2001; Underhill *et al.*, 2000). Both haploid markers also displayed regional restriction of ancestral haplotypes, suggesting that specific haplotypes developed in certain regions over time. This has become an important characteristic of the haploid markers that are used to study the demographic histories and migration routes of early populations.

2.3.3 Effective population sizes of early human populations

The large difference between the effective population size of the chimpanzee, which was estimated at ~35,000, and the first species of humans, estimated at ~10,000, suggests that the humans went through a population bottleneck during their divergence from the chimpanzee at ~5-6 million years ago (Kaessmann *et al.*, 1999). The effective population size of humans has been estimated at ~10,000 based on nuclear markers and at ~5,000 based on haploid markers (Hammer *et al.*, 1998; Ingman *et al.*, 2000). It is believed that *Homo sapiens* went through a large population expansion after the speciation event from *Homo erectus*, after which they dispersed across a large geographic region (Tishkoff and Verrelli, 2003).

The findings of separate studies of African and non-African populations indicated that the African populations had a larger effective size than the non-African populations (Tishkoff *et al.*, 1996; Jorde *et al.*, 1998; Hammer *et al.*, 1998; Rogers and Harpending, 1992), which could be explained by the hypothesis that the first human population that migrated out of Africa underwent a strong bottleneck, as indicated by the lower levels of genetic diversity within the non-African populations. Based on the findings of Ingman *et al.* (2000), the effective population size of 5,000 for a haploid population could be consistent with a bottleneck of about 1,000 females for about 3,000 generations (Relethford and Harding, 2001).

Another population expansion most probably took place with the development and spread of agriculture during the last 10,000 years (Cavalli-Sforza and Feldman, 2003). This event

was dated just prior to the early Bantu migrations to the southern regions of Africa and was most probably one of the reasons why these migrations took place.

2.3.4 Migrations and demographic changes of African populations

The extant genetic patterns that are observed in individuals of African origin have been shaped by large-scale movements of technology and culture driven by environmental changes, which have caused extensive gene flow between the African populations. Knowledge of the migration routes of African populations is therefore of critical importance to the interpretation of genetic patterns.

The genetic landscape of Africa has been influenced by migration events and the subsequent admixture between populations. The earliest fossil evidence of modern human existence, dated between 160 and 150 kya, was found in eastern Africa (White *et al.*, 2003) and is supported by genetic data suggesting that AMH had a predominantly single point of origin, which was located in the eastern regions of Africa, from where the dispersal of modern humans began (Salas *et al.*, 2002; Kivisild *et al.*, 2004).

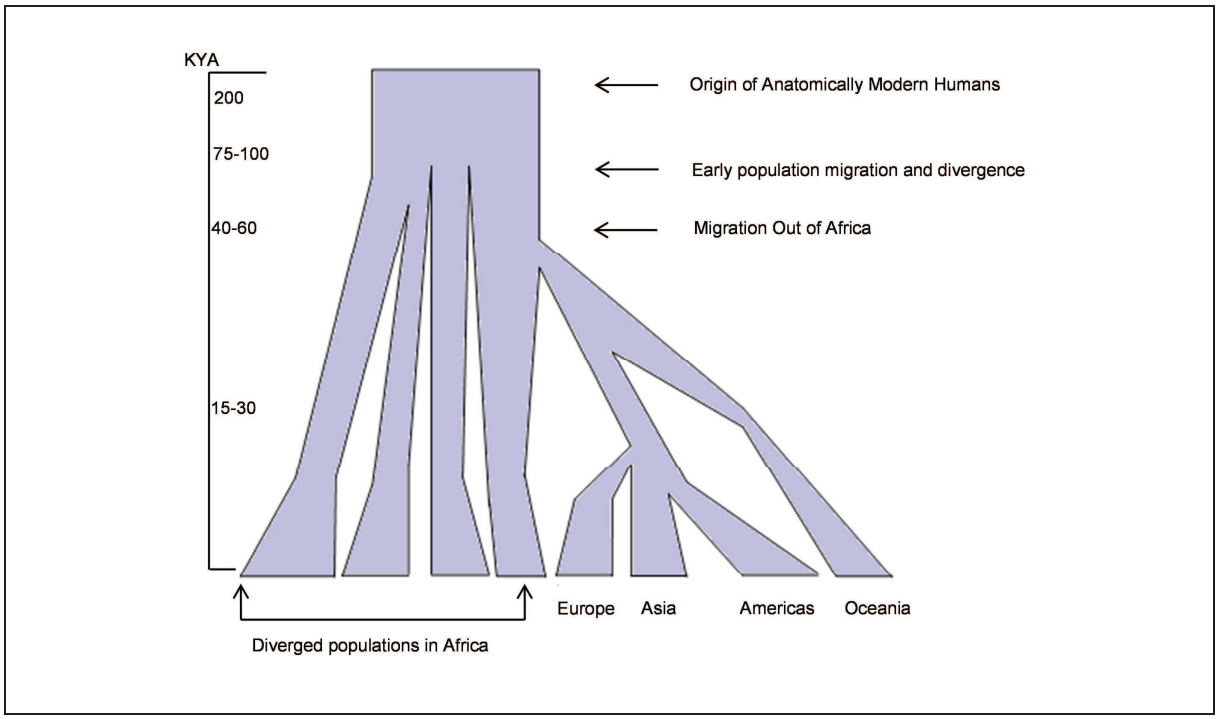
2.3.4.1 Migration Out of Africa

East Asia has been an important region in which to investigate human evolution and genetic diversity to determine the ancestral origins of the first AMH in that region. Autosomal (Tishkoff *et al.*, 1996), mtDNA (Yao *et al.*, 2002) and Y chromosome (Ke *et al.*, 2001) evidence supports the theory that the East Asian population arose from an African ancestral population that migrated between 60 and 40 kya from eastern Africa to the southern part of east Asia, from where some AMH populations migrated in a northerly direction to China, Siberia and America (Su *et al.*, 1999), while others migrated south to Australia and Papua New Guinea (Bowler *et al.*, 2003). Evidence exists that the migrations took place via two routes. The first was along the coast to south-east Asia, where the populations split into a group that migrated to the north and a group that migrated to the south (Stringer, 2002). The populations in the south reached Oceania between 60 and 40 kya and the northern migration reached China, Japan and eventually America at about 15 kya. The second route followed a central direction through the Middle East to central Asia, from where the rest of Europe was populated, as well as the eastern and north-eastern regions of Asia at about 40 kya (Cavalli-Sforza and Feldman, 2003).

Further proof that the humans that populated Asia and eventually Oceania, Europe and America originated from Africa, lies in the haplotypes in non-African populations that were also present in Ethiopian and Somalian populations. This suggests that the non-African populations contained genetic haplotypes that originated from northern African populations, which diverged from the rest of the sub-Saharan African populations and migrated out of Africa to populate the rest of the world (Quintana-Murci *et al*., 1999; Chen *et al.*, 2000). It is widely accepted that the entry of the first human populations from Africa to Europe and Asia was through the Levant (Cavalli–Sforza and Feldman, 2003).

The haplotypes that formed the earliest phylogenetic branches of the human tree remained in Africa, where the genetic variation displayed signals of population expansions 50,000 years ago, which has been interpreted as evidence of the growing hunter-gatherer populations. The lower level of genetic diversity that has been observed in non-Africans has been explained by a possible bottleneck that accompanied the migration out of Africa (Campbell and Tishkof, 2010).

Figure 2.1 Demographic history of early human populations



Demographic history of early *Homo sapiens* populations according to a timeline indicated on the left (in thousands of years (KYA)); the figure legs are representative of the diverged populations that originated from the first anatomically modern humans in eastern Africa; the divergence of early African populations into four (4) major lineages ~ 100 kya that migrated into different demographic regions; a subset of a north-east African population migrated out of Africa between ~40 and ~60 kya and was associated with a bottleneck event; this small population migrated into a broad geographical area to establish the populations in Europe, Asia, America and Oceania. Adapted from Tishkoff and Williams, 2002.

2.3.4.2 Early migrations in Africa

The sequence variation data of the human mitochondrial genome have been important in interpreting the origin and early migrations of the AMH and has suggested that the first human populations shared a common maternal ancestor who resided in eastern Africa from where the AMH split into two small genetically isolated populations about 100 kya (Salas *et al.*, 2002; Behar *et al.*, 2008). One of these populations migrated to the southern regions of Africa and the other migrated to the central and western regions of Africa (Behar *et al.*, 2008).

The ancient mitochondrial lineages that represent one of these populations are observed in the current Khoi-San populations of southern Africa and therefore it is believed that the Khoi-San populations represent the early genetic lineages of the population that migrated to the south and were isolated from the other population, which migrated to the central and western regions of Africa (Behar *et al*., 2008). The mtDNA data was supported by the findings of Y chromosome studies in which it was confirmed that the !Kung San population in the southern regions of Africa contained the most divergent genetic makeup of all populations in Africa (Hammer *et al.*, 2001; Underhill *et al.*, 2000). This was further supported by studies of classical polymorphisms and archaeological evidence that implied that the early Khoi-San ancestors migrated to the southern regions of Africa more than 10 kya (Tishkoff and Williams, 2002; Cavalli-Sforza and Feldman, 2003). The genetic data, however, do not preclude the possibility of a southern African origin of the AMH and that the Khoi-San population contains the lineages of a group that did not migrate further to the central and western regions of Africa (Henn *et al.*, 2011).

Archaeological evidence has suggested important changes in the subsistence strategies and cultural practices of human populations during the Middle Stone Age, which resulted in a population expansion around 75 to 55 kya (Mellars, 2006). It is believed that the population expansion was stimulated by the development of sophisticated stone tools and tools for navigation (Klein, 2000). These innovations would have increased food production, which would have allowed the populations to reside in a single region for longer periods during which greater expansion was more likely. Genetic signals of a population expansion event corroborated this theory and dated the migration event at between 86-61 kya (Salas *et al.*, 2002). Evidence of a climate shift from arid conditions to a wetter and more stable climate around that period, supplies the most feasible reason why the populations started to migrate towards the western regions of Africa (Scholz *et al.*,

2007). Further archaeological evidence suggeststhat a single population gained a cultural advantage over the other populations in the eastern African region at that time through the development of stone blade technologies, skin-working tools and cultural practices that involved the use of ornaments and red ochre (Mellars, 2006). These advantageous practices and the climate provided the impetus to begin the migration to the west.

The most pronounced population expansion signal was detected in one of the most common mtDNA lineages in Africa and dated back to between 31 kya and 25 kya (Salas *et al.*, 2002). It is believed that this population expansion was caused by the environmental conditions of the last glacial maximum (LGM), which caused the Sahara desert to enlarge and most of the forested areas of central Africa to become open savannah and woodland. This change in climate and environment most probably caused the populations to migrate towards the more central regions of Africa at that time (Mellars, 2006).

The LGM also caused climatic changes, which resulted in some of the regions of the African continent becoming more favourable for human habitation (González *et al.*, 2006). This period was followed by the Neolithic period, beginning at about 10 kya, in which major population migrations took place accompanied by large-scale cultural and technological movements that resulted in extensive gene flow between humans (Scheinfeldt *et al*., 2010). These dispersal events therefore had a great impact on the genetic variation of the populations at that time. The northern African populations developed novel technologies that were associated with a semisedentary subsistence lifestyle, which most likely resulted in population expansions (Bar-Yosef *et al.*, 1987). These populations further displayed genetic evidence of extensive gene flow with the populations of the Middle East (Semino *et al.*, 2004; Tishkoff *et al*., 2009). Archaeological evidence indicated that during the same period, the Sahel was an important corridor for bidirectional migration between the populations of eastern and western Africa (Cerny *et al.*, 2007).

Archaeology suggested that pastoralism originated in northern Africa at ~11 kya and that it spread with the migration of pastoralists to eastern and then to southern Africa (Henn *et al.*, 2008). The spread of pastoralism is an example of how genes and culture co-evolved over time in Africa. Agriculture and pastoralism were connected to specific dietary changes, which resulted in population expansions owing to the availability of food. Subsistence is therefore an environmental factor that could have a dramatic impact on the genetic diversity of populations based on the adaptations that humans make in terms of dietary changes through processes of positive selection. Sequence variants that would

have assisted humans to adapt to a change in diet from foraging to animal and plant products would have been increased in the pastoral and agricultural populations (Scheinfeldt *et al.*, 2010).

2.3.4.3 The Bantu migrations

The Bantu expansion refers to one of the largest population migrations in recent African history about 5,000 years ago, which entailed a complex transmission of culture, language, technology and genes to the southern regions of Africa. Climate changes caused the Bantu-speaking populations that were located in the western-central regions of Africa near north-western Cameroon and the southern parts of Nigeria to migrate across the central region of Africa along eastern and western routes to the southern regions of Africa (Salas *et al.*, 2002). These migrations entailed large-scale movements of farmers with knowledge of agricultural traditions that were well suited to the climate of the sub-Saharan regions of Africa and were probably related to the spread of agricultural technologies and the beginning of the development of iron technologies (Vansina, 1995).

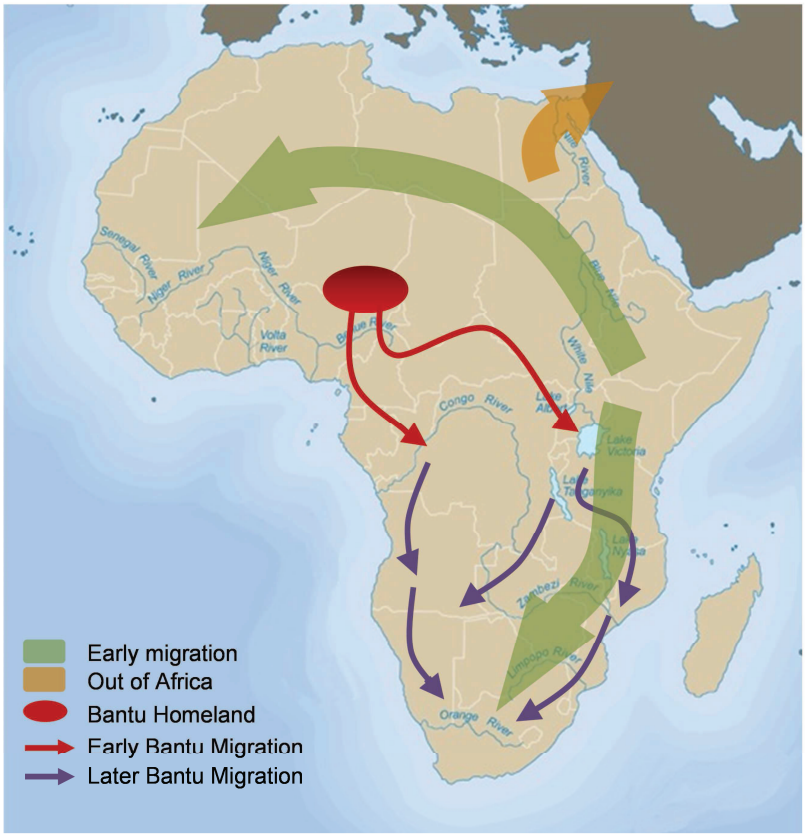
The western route followed the wet coastal regions of the central African forests of Africa toward the southern regions, as opposed to the second migration, which moved towards the eastern interlacustrine region from where it followed a southward route along the coast and then through the central internal regions (Castri *et al.,* 2009; Coelho *et al.*, 2009). The western route therefore resulted in the habitation of the equatorial rainforest by early African populations at ~3,5 kya and the eastern route resulted in a group of African populations that settled in the interlacustrine region, which was located in the modern-day country of Uganda (Pereira *et al.*, 2001). These Bantu-speaking population movements resulted in the isolation of or admixture with neighbouring ancient populations of those regions, namely the Pygmies and the Khoi-San populations (Cavalli–Sfroza and Feldman, 2003). This resulted in a spread of the Bantu-speaking languages and culture across the sub-equatorial regions of Africa, as well as extensive gene flow between these populations to establish a modern population that consists mostly of Bantu-speaking populations and widespread evidence of early Bantu culture and traditions. The eastern core of populations that were located in the interlacustrine region migrated to the southern regions of Africa via two routes. One group migrated along the Ruvuma River to the modern-day KwaZulu-Natal in South Africa and another group migrated along the shores of Lake Malawi through the current eastern Zimbabwean regions and to the current northern Limpopo province of South Africa (Pereira *et al.*, 2001).

The Bantu migration resulted in the widespread use of Bantu languages, culture and genetic variation harboured by the Bantu speakers that were ancestrally connected to the early origins of the Bantu speakers. In contrast to the expected homogeneity of the Bantu-speaking genetic background due to the large presence of Bantu-speaking populations in the southern regions of Africa, the genetic compositions of the different Bantu-speaking populations differed greatly (Pereira *et al.*, 2001; Salas *et al*., 2002). This was ascribed to the fact that the Bantu migration was not a single continuous migratory event but consisted of waves of migrations via different routes and at different times. The effects of the Bantu migrations are therefore visible in the genetic diversity of the sub-equatorial African populations, which left genetic footprints in many of the extant African populations (Pereira *et al*., 2001; Salas *et al.*, 2002; Wood *et al*., 2005).

Genetic data contributed to the theories about the major dispersal of the Bantu speakers to the southern regions of Africa. MtDNA markers suggested that most of the Bantu lineages could be associated with a migration from the central/western regions of Africa through the central region towards the southern regions, as opposed to a western route (Soodyall *et al.*, 1996, Pereira *et al.*, 2001; Salas *et al.*, 2002). The western route has, however, not been excluded as a possible route that some of the populations from western Africa followed to modern-day Angola and Namibia and it was postulated that the western migration took place prior to the migration via the eastern route (Coelho *et al.*, 2009).

Comparisons between studies of mtDNA and Y chromosome markers suggested that the maternal and paternal histories of the Bantu migrations differed (Wood *et al.*, 2005). The levels of Y chromosome variation were in general lower than the levels of mtDNA variation and the haplogroups were generally associated with the Bantu expansion, whereas the mtDNA lineages also included lineages that had been present in the region prior to the Bantu expansion (Pilkington *et al.*, 2008). Patrilocality and polygyny were the most likely reasons for these findings (Berniell-Lee *et al*., 2009).

Figure 2.2 Population migrations within Africa



Map of Africa, adapted from http://exploringafrica.matrix.msu.edu/ accessed on 14 November 2011. The migration routes indicated by coloured arrows are explained by the legend. Early migration = early *Homo sapiens* migration at ~100 kya from eastern Africa via two routes; the western route that populated the central, western and northern regions of Africa and the southern route; which populated the southern regions of Africa with the hunter-gatherers (Khoi-San speaking populations); Out of Africa = the route that a small population took between ~60 kya and 40 kya to migrate out of Africa and populate the rest of the world; Early Bantu migration = the great Bantu migration at ~4 kya, which started in the Bantu homeland of north-western Cameroon and Nigeria via eastern and western routes; Later Bantu migrations at ~2 kya via the eastern route through the interlacustrine region through Zimbabwe to southern Africa and via the tropical rainforests towards Angola and Namibia. From Soodyall *et al.*, 1996; Pereira *et al.*, 2001;Salas *et al.*, 2002; Castri *et al.*, 2009; Coelho *et al.*, 2009.

2.3.5 The prehistory of southern African Khoi-San speaking populations

The term “Khoi-San” refers to the hunter-gatherer and herder communities of southern Africa and collectively also to the language and culture shared by many of these populations (Mitchell, 2010). These populations are further also referred to as San, Hottentots, Khoe and Bushmen. Studies of mtDNA (Chen *et al.*, 2000), Y chromosomes (Hammer *et al.*, 2001) and autosomal chromosomes (Tishkoff *et al.*, 2009) have all reported that the Khoi-San speaking populations of southern Africa display the most diverged lineages of any populations in the world. Most of the haplotypes observed in these populations are unique to the Khoi-San speakers and some are connected to other ancient hunter-gatherer populations, such as the Sandawe and Hadzabe of Tanzania and

the Pygmies of central Africa (Gonder *et al.*, 2007; Tishkoff *et al.*, 2007, Quintana-Murci *et al.*, 2008). It is believed that the modern-day Khoi-San speakers of southern Africa diverged from the other modern humans ~90 kya (Behar *et al.*, 2008) and that they followed an isolated existence from other populations and resided in eastern Africa, north of the current Zambezi River (Mitchell, 2010).

A pre-Iron Age movement of livestock and most likely also of humans is suggested to have taken place about 2 kya into south-central Africa (Henn *et al.*, 2008). The herders and hunter-gatherers then mixed and adopted the pastoral lifestyles that came with the migrating Bantu-speaking food producers to the south, from where these hunter-gatherers and/or herders migrated south and introduced cattle-keeping, sheep-keeping and pottery to the other Khoi-San speaking populations on their way. The migration culminated in the Western Cape province of South Africa where the Khoi-San speaking populations most probably came into contact with the Europeans that had arrived in the Cape (Sadr, 1998). The current coloured population that resides in the Western Cape displays clear genetic signals of admixture with European lineages (Quintana-Murci *et al*., 2010).

The Khoi-San generally speak languages that contain a large number of clicks, which are referred to as click languages and these vocalisations are useful in distinguishing between the different Khoi-San populations of southern Africa. Three click-language families have been identified in southern Africa, namely the Tuu, Ju and Khoe, also referred to as Southern, Northern and Central Khoi-San (Barnard, 1992) or!Ui-Taa, Ju and Khoe. The Ju and Tuu speakers are traditionally regarded as hunter-gatherers and include the !Kung, whereas the Khoe speakers are regarded as both herders and hunter-gatherers. Some of the Khoe-speaking populations are pastoralists and include the Nama populations of Namibia and the Namaqualand region in South Africa, as well as the Khoekhoen of the Cape (Mitchell, 2010).

Genetic data suggest that the Khoi-San speaking populations of southern Africa did not necessarily have the same ancestral origins. This hypothesis was based on evidence that some of the Khoe-speaking populations displayed genetic relatedness with each other, such as the Nama and the Ju≠Hoan, whereas other Khoe-speaking populations such as the Dama and the Khwe displayed genetic ancestry with other African populations, such as the Sandawe from Tanzania (Henn *et al.*, 2008).

The Kwadi language dialect was spoken by a group that is now extinct and was linked to the Khoe speakers. Some of the Khoe-Kwadi language family of the Khoi-San populations of southern Africa resided in Botswana and were classified by Barnard (1992) into four divisions of Khoe-speaking Bushmen, namely the Western Khoe Bushmen, which were in close contact with the !Kung, the Central Khoe Bushmen, which included the G/wi and G//ana of the Kalahari Game Reserve of South Africa, the Northern Khoe Bushmen that resided in the Okavango and the Eastern Khoe Bushmen that resided in the eastern regions of Botswana. The Eastern Khoe Bushmen linguistically constituted the Shua and the Tshwa language groups and were influenced by the Tswana culture (Barnard, 1992). The Khoe-Kwadi family also differed from the other Khoi-San-speaking populations in that they were taller and had darker skins; they combined cultivation with herding, hunting and gathering (Mitchell, 2010).

2.3.6 The prehistory of southern African Bantu-speaking populations

More than 400 Bantu languages are spoken in the sub-Saharan regions of Africa, which constitute a specific branch of the Niger-Congo language group, and are believed to have originated in the western-central regions of Africa about 4 kya. Thereafter these languages were dispersed in southern Africa through the early Bantu migrations via an eastern and a western route (Vansina, 1995). Therefore the Bantu-speaking populations of the current southern African region can either be classified in a linguistic category that is related to the recently derived eastern Bantu languages or from the more anciently derived western African Bantu languages (Vansina, 1995).

The transmission of pastoralism to the southern regions of Africa most probably occurred as a result of population movement from eastern Africa as the Bantu migrations entered the southern-central regions of Africa with their livestock. The alternative model, which stated that pastoralism reached southern Africa through cultural diffusion, was refuted by Y chromosome and mtDNA studies, which confirmed that the Bantu-speaking populations of eastern Africa underwent a demic movement to the southern regions of Africa (Tishkoff *et al.*, 2007; Henn *et al.*, 2008). The model for demic diffusion was further supported by the cultural artefacts of the late Iron Age that were discovered in the southern regions of Zambia (Vansina, 1995). These artefacts were connected to cattle herding and suggested that the Bantu-speaking agriculturists came into contact with the Khoi-San speaking local groups and exchanged cultural artefacts and lifestyle knowledge. Genetic studies also

suggested that gene flow occurred between the southern hunter-gatherer populations and the eastern pastoral populations between ~1,2 and 2,7 kya (Henn *et al.*, 2008).

Cattle were introduced into Angola via an eastern route from northern Africa, where the major component of the Iron Age source populations of south-western Africa resided in the Congo, as is evident from the large component of Congo Basin affiliated languages that are spoken in Angola and Namibia (Vansina, 1995). Genetic data suggest that the same type of admixture between the Bantu-speaking agropastoralists and the Khoi-San speaking populations took place, as was the case in eastern South Africa (Coelho *et al*., 2009).

Evidence of ceramics and pottery discovered in South Africa indicate Nguni Bantu settlements in KwaZulu-Natal and the eastern Cape provinces of South Africa about 800 years ago and Sotho-Tswana Bantu settlements in the northern province of Limpopo about 700 years ago. Archaeological evidence of ceramics and pottery styles that were discovered in eastern Zambia and Tanzania suggested that both of these populations originated from eastern Africa (Huffman, 1989). The nine (9) Bantu languages that are spoken in South Africa are Zulu, Xhosa, Pedi, Tswana, Southern Sotho, Tsonga, Swati, Venda and Ndebele, of which only the Swati and Ndebele languages can be related back to western Africa. The seven other Bantu languages spoken in South Africa are therefore connected to eastern African languages (Lane *et al.*, 2002). The Nguni and Sotho/Tswana languages especially share some linguistic features with the eastern African languages, as opposed to the more distant Shona and Bantu languages of northern Namibia that present no shared features with the eastern African Bantu languages (Huffman, 2002). Genetic studies performed on individuals that belong to these seven language groups did not display large genetic differentiation and suggested that the Bantu-speaking populations of South Africa shared common ancestors that most probably originated in eastern Africa (Lane *et al.*, 2002).

2.3.6.1 The history of the Tswana-speaking populations of southern Africa

As mentioned in the previous section, the Sotho-Tswana Bantu-speaking populations settled in the northern province of Limpopo about 700 years ago (Evers and Van der Merwe, 1987). This is evident from the discovery of Moloko style pottery that is widely regarded as typical of the Sotho-Tswana speakers (Evers and Van der Merwe., 1987). It is believed that the Tswana-speaking Bantu population reached the north-eastern and

south-western regions of the Vaal River in South Africa, from Botswana, between 700 and 800 years ago (Boeyens, 2003). Huffman (1989) hypothesised that the Moloko style had its origin in eastern Africa and that the migration to southern Africa was in tandem with the Nguni-speaking populations, which originated from Tanzania. The period in which the Sotho-Tswana and the Nguni people migrated to southern Africa coincides with the closing phase of the Medieval Warm Period, which lasted from *Anno Domini* (AD) 900 to AD 1290 (Huffman, 1989) and which most probably caused a severe drought in eastern Africa. This was also the time in which the subtropical southern regions of Africa experienced higher rainfall and were therefore more inhabitable than their initial homeland.

The four (4) clusters within the Sotho-Tswana-speaking group that resided in South Africa i.e. the Hurutshe, Kgatla, Rolong and Fokeng, had separate origins. The Hurutshe originated in modern Botswana and migrated to the Marico; the Kgatla originated in the region of the current town of Rustenburg and were the precursors for the later Pedi populations; the Rolong originated near the town of Zeerust and were later replaced by the Hurutshe, and the Fokeng resided at Ntsuanatsatsi Mountain near the southern highveld and later migrated south-east across the Vaal River (Huffman, 2002). The Hurutshe was, however, regarded as the most senior genealogical tribe of the various Tswana tribes of South Africa (Boeyens, 2003).

The next migration of the Sotho-Tswana-speaking populations was connected to the onset of the Little Ice Age, which occurred at about AD 1300 and caused the further migration of the Tswana-speaking populations into regions that were previously inhabited by the first Bantu-speaking farmers in South Africa (Boeyens, 2003). By AD 1500, the Sotho-Tswana-speaking populations had reached central Marico in the North West province of South Africa and settled near Tswenyane (Boeyens, 2003). A change in their lifestyles occurred at the end of the Little Ice Age at about AD 1700; archaeological evidence points to the increased use of stone for building purposes and settlements on hilltop sites rather than at the foot of hills. This period also coincided with severe drought, political instability and population migrations in South Africa. The instability was worsened by the infiltration of the politically unstable Pedi-speaking populations and the incursion of the Ndebele populations into the region north of the Vaal River (Breutz, 1989). Based on the conflict between chiefdoms, military stress and defensive strategies, and population growth, the Tswana populations were aggregating in large towns in the Marico region during AD 1800 (Huffman, 2002).

By this time the Tswana-speaking populations were relying on maize as a staple, which became extremely scarce during the severe drought that occurred in the period between 1790 and 1810. The Tswana populations were therefore not only subject to conflict with other Bantu-speaking populations, but were also starving because of an imbalance between the large number of Tswana-speaking individuals and the limited resources they had as a result of aggregating in large settlements in this time of *difaqana* (Huffman, 2002). The pressures felt by the respective Tswana tribes eventually resulted in internal tribal wars, which led to a major split in the Hurutshe tribe and subsequent fragmentation of the Tswana-speaking populations of that time.

The Tswana people started migrating to Botswana around AD 1700 and settled in a region surrounding the Kalahari Desert at about AD 1800, which was inhabited by the Khoi-San speaking hunter-gatherer populations. The current six Tswana tribes, the Bakwena, the Bangwaketse, the Batawana, the Balete, the Barolong and the Batlokwa, were formed in this region during that period. The Khoi-San people that were conquered and those that lost their land were incorporated into the Tswana tribes as slaves and the Khoi-San people that managed to share the region with the Tswana tribes were levied by the Tswana chiefs. Little evidence is available about the period that followed the initial settlement of the Tswanas in the Kalahari, but it is known that Tswana males married Khoi-San women of the region and that the populations lived peacefully together, according to evidence of shared hunting-gathering practices between the Tswana tribes and the Khoi-San hunter-gatherers (Osaki, 2001).