Chapter 1: Introduction
1.1 Genes in space and time: Statistical phylogeography

Evolution works at multiple levels of biological organisation. Understanding the spatial distribution of genetic variation and its evolution over time is essential to understand how populations evolve. The present distribution and abundance of genetic variation in a population, however, is also the consequence of past events. Genetic variation in extant populations can thus be effectively understood by investigating both their spatial and temporal dimensions across their geographic range.

1.1.1 Patterns of genetic variation in space

Individuals or gametes carry genes through space by their dispersal. If individuals disperse from one population to another and reproduce successfully, this will lead to gene flow between the populations, whereby both the populations would share many more similar alleles than just by chance alone. Gene flow has long been considered a significant evolutionary force that shapes populations (Slatkin 1987). As a homogenising force, gene flow resists the differentiating effects of mutation and natural selection on populations. Populations defy isolation through gene flow and genetic homogeneity spreads through the entire species range. In contrast, barriers to gene flow isolate populations, which eventually differentiate from one another over time, as a result of the accumulation of mutations, effects of natural selection and genetic drift. This balance between gene flow and other evolutionary forces maintain the genetic equilibrium of populations. Occasional shifts in this balance, due to historical events, change the evolutionary trajectory of populations, which then strive to achieve a new equilibrium of forces over a multitude of successive generations.

Alternatively, a population, as a whole, may also expand its distribution through migrating individuals who occupy new territories in adjacent areas. Interestingly, such expansions may happen in two very different ways. A population can grow in overall size and such a changing distribution of purely demographic nature can promote waves of new alleles in the forefront of the expanding edge of the population (Excoffier et al. 2009). In contrast, if populations expand only in range, not necessarily in size, then such spatial expansions also would affect the pattern of genetic variation albeit very differently from its purely demographic version. Theoretical studies have suggested that range expansions can actually generate allele frequency gradients within populations, boost the surfing of rare alleles into newly occupied areas and thus inducing
a dynamic re-structuring of the population (see Excoffier et al. 2009 for details). In effect, pure
demographic or range expansions, fuelled by long-distance gene flow, shape the distribution of
genes in space and these patterns accumulate over many generations to give rise to occasionally
very different population dynamics than that exhibited originally.

1.1.2 Patterns of genetic variation in time

The movement of genes through time is usually propelled vertically by reproduction (but see
Syvanen 2012 for a review on horizontal gene transfer). As genes move from one generation to
the next, new genetic variants may arise through mutation while pre-existing or newly arisen
mutations are lost through natural selection or random genetic drift. Mutation rates vary among
different genetic loci but are thought to be relatively constant over time for any given locus.
Furthermore, a large proportion of an organism’s genome is considered to be more or less
selectively neutral (Ohta 1992, but see Nielsen 2005). Thus, the number of neutral loci in an
isolated finite population is a balance between the mutation rate and the amount of genetic drift,
where the latter is a function of the effective population size (Kimura and Crow 1964). The
effect of genetic drift will be higher on a population with smaller effective population size than
on one with a larger effective size. Consequently, changes in effective population size will lead to
a change in the number of neutral genes in a population through genetic drift.

There is, moreover, a direct connection between effective population size and the dispersal of
individuals, manifest through the density achieved by the population. Additionally, dispersal is
often associated with population density, with higher densities generally promoting high
individual dispersal rates. Dispersal can, therefore, be both positively and negatively affected by
population density (Travis et al. 1999). In cases where dispersal is positively dependent on
density, a reduction in population density through a reduction in its size may lead to a decrease in
dispersal. This, consequently, would decrease the rate of gene flow among subpopulations,
which, in turn, would increase the effect of genetic drift within each of those subpopulations.
Finally, genetic drift may lead to lowered fitness of the individuals and subsequently, a further
decrease in population size and density (Lande 1988).
1.1.3 Statistical phylogeography: Integrating population genetic structure and demographic history

Phylogeography is a very young field of evolutionary biology that studies genealogies within or between populations to understand the spatial patterns of genetic variation (Avise 1987). This field incorporates both the dimensions of space and time as it serves to be “a field of study concerned with the principles and processes governing the geographical distributions of genealogical lineages, especially those at the intraspecific level” (Avise 1998). The main contribution of the field has so far been to elucidate the different factors that affect the population genetic structure and divergence of lineages (Knowles and Maddison 2002). Typically, phylogeographic inferences are derived by studying the reconstructed genealogical histories of individual genes sampled across many populations. Reconstructing the past, however, is an exercise fraught with difficulties as many lineages may have been lost just by chance alone or due to the occurrence of historical events such as population size changes, vicariance and migration. Hence, the analysis and interpretation of phylogeographic data usually involve inputs from molecular genetics, population genetics, phylogenetics, demography, ethology and historical geography, which effectively make it an integrative field of study (Avise 1998).

More recently, however, the field of phylogeography has experienced a paradigm shift in the form of integrating phylogeny with population genetics, as was first proposed by Avise (2000). This has been possible mainly due to two developments, the first being the use of multilocus data instead of only that from a single gene to address phylogeographic problems, thus providing higher resolution into past events. Secondly, in the last ten years, there has been a surge in the development of statistical approaches based on the coalescent method (Beaumont et al. 2002; Beaumont 2010). Both these developments have made it possible to estimate demographic parameters and test population genetic hypotheses, effectively giving birth to the field of statistical phylogeography (Knowles and Maddison 2002). Under this approach, statistical models are built to test specific hypotheses under a Bayesian and/or a likelihood-based framework using the coalescent theory. Indeed, under the Bayesian/likelihood-based schemes, each competing hypothesis can be evaluated by applying the data to each model relative to the other models, by way of different decision theoretical methods such as Bayes factors or likelihood ratio tests. Alternatively, a model can be treated as a null hypothesis, whereby the probability of observing a simulated dataset more extreme than the observed data is calculated under the assumption that the null hypothesis is true. Under either approach, however, none of the competing models is
considered to be real, but only a useful approximation of the real demographic history of the population; it is, therefore, most useful if it is somewhat robust to a violation of the model assumptions (Wakeley 2004). Over time, a refinement of these models is expected to reveal the demographic history of the study population more accurately. This approach has, nevertheless, effectively allowed us to extend our abilities to delve into demographic questions that could not be addressed even a decade ago. Consequently, statistical phylogeography integrates investigations into the structuring of populations with those into the past processes such as change in population size, migration rates and divergence times that may have shaped population structure. These past processes cumulatively form the demographic history of a species (Knowles 2009).

1.1.4 Factors affecting the phylogeography of species

Many factors may affect gene flow across populations through the movement of individuals and thus, in effect, the phylogeography of a species. Climate change is known to have altered habitats for species populations many a times in the past. Decrease in available habitat is unlikely to have a large impact on gene flow, as it would primarily involve a directional movement of individuals into patches already inhabited by conspecific individuals. An increase in available habitat, however, would have a much larger impact, as empty habitats are easy to occupy. Due to constantly changing environments, species have historically undergone many changes in their population size. Changes in historic climatic patterns have been responsible for major changes in the population size of species worldwide. Many species have, for example, gone through population size contractions during the Pleistocene glaciations and expansions during the interglacial warm periods, particularly in Europe and North America (Hewitt 1996). Historical cataclysmic events such as asteroid impacts or volcanic super-explosions have also been hypothesised to have caused drastic reductions in population size in many taxa in the past (Schulte et al. 2010). In more recent times, human activity has led to overexploitation of species, changes in the amount of available habitat and introduction of new competitors and predators in the environment. On a smaller scale, genetic variation may itself affect changes in population size through phenomena such as inbreeding depression, loss of genetic diversity and the accumulation of mutations (Frankham 2005).

Another broad-scale factor is constituted by geographical barriers, which are also known to strongly influence the spatial movements of individuals. Such barriers range from oceans and
mountain ranges to small streams or patches of low-quality habitat depending on the intrinsic dispersal ability of the organism. Biotic factors, as, for example, competition and predation, can also have an effect on individual movements as presence of competitors or predators may act as geographical barriers to dispersal. More recently, a cultural preference for certain habitats has been found to restrict gene flow between populations in some marine mammals (Hoelzel 2009).

In conclusion, the distribution of neutral genetic variation in a population or species is governed by the movement of genes through space and time. The present geographic patterns (population genetic structure) displayed by a taxon are the consequence of past change in the size of its populations (demographic history), and past and present gene flow, which are, in turn, affected by a multitude of factors, both biotic and abiotic. I will now attempt to briefly review the existing literature on phylogeographic studies, although for a more detailed review of the field, readers are directed to Hewitt (2004).

1.2 Phylogeography: The state of the art

The strength of statistical phylogeography lies in its capacity to make inferences about temporal changes in the physical and biotic environments of a population using contemporary genetic data alone. The employment of historical data, in addition, makes the approach even more robust. This approach, thus, has already made its presence felt across different branches of evolutionary biology, theoretical and applied alike, in spite of being a very young (phylogeography, 25 years; statistical phylogeography, 12 years) field of science (see Beheregaray 2008 and references therein).

It is clear from recent reviews (Beheregaray 2008; Knowles 2009) that the field has seen a surge in studies across the globe. But the distribution of studies is far from equal across geographical regions or taxa. Beheregaray (2008) observed that vertebrates were the subject of more than half the reviewed studies while mammals accounted for 21% of all the investigations. In contrast, invertebrates and microorganisms were the least studied of all groups. Among geographical regions, systems from the northern hemisphere were disproportionately well represented (77%) among all the taxonomic groups (Beheregaray 2008). Several regions from developing countries within the northern hemisphere, as, for example, Sri Lanka, mountains of central Asia, Irano-Anatolian region, Himalayas, mountains of southwest China and the Philippines, however, were
found to be particularly data-deficient (Beheregaray 2008). Many of these areas are considered as hotspots of biodiversity and are situated in the tropical regions of the planet. These are also, rather unfortunately, areas with some of the highest rates of biodiversity loss (Mittermeier et al. 2000).

1.2.1 The higher latitudes: The Arctic regions

Most phylogeographic studies from the northern hemisphere have taken place in the temperate regions but recently a number of species including mammals, birds, fish, crustaceans and plants have been studied from the Arctic region (Avise 2000; Hewitt 2000). During the ice ages, the greatly extended Arctic ice sheets forced most of the resident species south. According to Hewitt (2004), large areas of northeast Asia and the northwestern corner of North America were covered in permafrost but not glaciated during the Last Glacial Maximum (LGM) and, as a result, acted as refugia (for example, Beringia) to many of these species populations. Further, genetic evidence suggests a relationship between mobility and the intensity of genetic structure among different species from the region with the less mobile species showing relatively more structure than do the mobile species, particularly during the late Pleistocene ice age (Hewitt 2004).

1.2.2 Mid latitudes: The temperate regions

Temperate species are considered to inhabit habitats between 20°C and 60°C, and broadly distributed according to their cold tolerance, with more tolerant species being more northerly in their distribution (Hewitt 2004). During the ice ages, these two groups of species were thought to have retreated to refugia further south of their present-day distributions (Hewitt 2004). The major refugia for these species during the ice ages were believed to have existed in the Balkans, and the Italian and Spanish peninsular (Hewitt 2000). Overall, among habitats in temperate regions like Europe and North America, there seems to be more diversity in the southern part, where most of the refugia were thought to have accumulated over many ice ages, than the in the north, where most of the genetic variation was, perhaps, lost during postglacial colonisations (see Hewitt 2004 for a review).
1.2.3 The lower latitudes: The tropical regions

Most of species-rich Africa, South America, south- and south-east Asia, and northern Australia form the tropical regions of the Earth which, unfortunately, largely remain extremely understudied (Hewitt 2004; Beheregaray 2008). Some phylogeographic studies from American and Australian rainforests and a few from Africa and Asia indicate that there is great genetic diversity produced by a complex history, often diverging in the Pliocene period (Hewitt 2000; Moritz et al. 2000). A few recent studies of large savannah mammals from the dry tropics of Africa suggest the presence of three major refugia, namely the west, east and south, during the LGM, when the tropical rainforest reduced considerably in size due to climatic fluctuations (Hewitt 2004). It is noteworthy, however, that species across the tropics exhibit more species-specific phylogeography than they do features common across taxa. Such variation in population structure is possibly the reflection of their highly variable biology, the effect of specific microclimates and consequently, their more complex evolutionary history. Hewitt (2004) thus suggests that “(T)he richness and diversity seen at the species level is multiplied by that within species, and more studies are needed on biotas from such habitats to properly describe and understand them”.

1.3 Genetic consequences of primate social organisation

Primates, as a group, are significantly different from most other mammalian species in certain crucial aspects of their biology. Primates, for example, more than any other mammalian order, tend to form stable, bisexual and sometimes very large social groups, where dispersal is usually by individuals of one sex (Schülke and Ostner 2012). They also have long lifespans and generally possess slow life histories (van Schaik and Isler 2012). As a result, closely related or familiar non-kin individuals often associate for long periods of time to form stable social groups.

“Primate groups thus often comprise multiple, overlapping generations and cohorts of individuals linked to one another and to members of other social groups by complex pedigree relationships” (Di Fiore 2012).

Such generality, however, is equally challenged by tremendous flexibility and the resultant variation in social interactions between individuals within a group or between groups of the same
species. This intrinsic variability in behavioural interactions in primates makes it almost impossible to typify the group (Strier 2005). As a result, the population genetic structure of a primate group usually emerges from the complex interactions between several aspects of the social system such as sex-biased dispersal, dominance hierarchies, reproductive skews, patterns of group fissions and fusions, and relatedness within and between groups in a population, unlike the traditional view of how population structure is usually shaped (Fig. 1.1). Indirectly, the decisions made by individuals regarding mate choice, dispersal or the identity of groups they ultimately join following group fission or dispersal could be sensitive to their own relatedness to their social partners and, in turn, may influence the genetic structure of the social groups and populations themselves (see Di Fiore 2012 and references therein).

**Figure 1.1** Alternative views of primate population genetic structure. The classical approach treats a population as a conglomerate of many randomly mating, semi-isolated subpopulations (or demes), which are connected to one another by gene flow. More recently, this simplistic model has been made relatively more realistic by introducing directionality in gene flow (isolation-by-distance versus stepping-stone models) or by changing rates of migration (equal gene flow versus source-sink dynamics). The alternative social structure approach considers the presence of many social units (or groups) within each subpopulation. These units may vary in age and sex composition, as well as in mating behaviour as a result of independent individual lifehistories (modified from Sugg et al. 1996).
These influences are, however, in addition to the more general stochastic events such as loss of animals due to hunting, disease or natural disasters, or stochasticity in variables such as birth or mortality rates, age structure and sex ratios, which directly influence all organisms and thus will not be discussed here. Interested readers are referred to Hartl and Clark (2007) for detailed discussions on the effect of these stochastic factors on natural populations. It is to be noted, however, that the interactions of these social factors with the population genetic structure is expected to be bidirectional as individual decisions to disperse or reproduce may very well depend upon the genetic structure of the group itself. Thus, unravelling the genetic structure of primate groups to understand primate social system is as important as is the reverse (Di Fiore 2012). Application of the social structure approach in population genetics, however, requires study populations where individuals are identified and have been studied over many generations. Unfortunately, very few phylogeographic studies have been conducted on wild primate populations that have been studied over long periods of time (for example, Altmann et al. 1996).

### 1.4 The Cercopithecidae

The family Cercopithecidae, commonly known as Old World monkeys (OWM), encompasses at least 21 genera and thus represents one of the largest and most diverse of all primate families (Groves 2001; Disotell 2003). OWMs can further be divided into two ecologically and morphologically distinct subfamilies, namely, Cercopithecinae or the cheek-pouched monkeys and Colobinae or the leaf-eating monkeys (Delson 1992; Groves 2001). According to fossil records, these two subfamilies diverged from a common ancestor during the Miocene period (Oates and Davies, 1994).

The subfamily Cercopithecinae includes the baboons, the macaques, guenons and the mangabeys. While most cercopithecine monkeys are limited to sub-Saharan Africa, the macaques range from western Asia to the far eastern parts of the continent, with a single species restricted to the northern tip of Africa.
1.5 The macaques

One of the most successful nonhuman primate groups is represented by the genus *Macaca*, commonly known as macaques. Among the extant primates, macaques, with 22 species (Thierry 2007), occupy a geographical range that is smaller than only that of humans. Their distribution ranges from equatorial to temperate ecosystems, from evergreen primary forests to grasslands, mangrove swamps, semi-deserts and even areas of dense human habitation (Fooden 1982; Richard et al. 1989).

The macaques also exhibit high diversity in their behaviour. The diversity is manifest in their patterns of affiliation, aggression, dominance, nepotism, maternal behaviour and socialisation, which, in turn, infuse a high degree of variability in their social organisations. Macaques usually form female-bonded, multimale-multifemale groups containing both adult males and females, and their dependent offspring. Females are also known to be generally philopatric where females stay with their natal group for life. In addition, females form kin-bonded subgroups or matrilines within their natal groups and most males usually disperse after attaining sexual maturity (Thierry et al. 2004). Macaque societies are largely promiscuous with both sexes being able to exercise a certain degree of mate choice, except in a few despotic societies represented by species like the lion-tailed or pig-tailed macaques, in which each group usually harbours one or two adult males that are able to monopolise receptive females and restrict the access of other males to them.

In terms of their wide distribution, large population size and the range of habitat types exploited, macaques have achieved outstanding evolutionary success among primate groups. Although their distribution and numbers have reduced drastically since the Pleistocene, when macaques reached the peak of their evolution, the ecological adaptability and behavioural flexibility of the genus has surely contributed to their colonising success and the ability of some of the species to thrive in habitats that have or are currently undergoing drastic modification by humans.

1.5.1 Macaque phylogeny and taxonomy

Evolutionarily, as described above, the genus *Macaca* represents one of the most successful radiations of any anthropoid primate group. The 22-odd macaque species are widely distributed in southern and south-eastern Asia, with the exception of the Barbary macaque in northern Africa (Fooden 1982). While the earliest known fossils of the genus date to around 5.5 million
years before present, mainly from North Africa and Europe, macaques are evolutionarily of more recent origin in Asia (Delson et al. 2000). Molecular estimates of the divergence times, based on complete mitochondrial genomes and calibrated with several well accepted fossil divergent times, suggests the divergence of the macaques from other members of the tribe Papioninini approximately 9–10 million years ago (mya, Raaum et al. 2005). It was proposed subsequently, based on ancillary molecular and fossil evidence, that following this divergence, macaque ancestors probably entered Eurasia through northeast Africa approximately 5 mya. Subsequently, the Asian macaque lineage diversified into three to four species-groups less than 3 mya (Tosi et al. 2003). In spite of the narrow window of recent evolutionary radiation of the genus, macaques are still unparalleled among the extant primate genera by the sheer number of species that it contains and the remarkable ecological and behavioural adaptability displayed by these species.

Species of *Macaca* have been variously separated into several species-groups based on morphological features (Fooden, 1976; Delson, 1980; Fa, 1989; Groves, 2001). Initially, Fooden (1976) thus classified macaques into four species-groups on the basis of their penile morphology:

1. the *silenus-sylvanus* group, comprising Barbary macaques *M. sylvanus*, lion-tailed macaques *M. silenus*, pig-tailed macaques *M. nemestrina* and Sulawesi macaques *M. nigra*;
2. the *fascicularis* group, including long-tailed macaques *M. fascicularis*, rhesus macaques *M. mulatta*, Japanese macaques *M. fuscata*, and Formosan rock macaques *M. cyclopis*;
3. the *sinica* group, including toque macaques *M. sinica*, bonnet macaques *M. radiata*, Assamese macaques *M. assamensis* and Tibetan macaques *M. thibetana*;
4. the *arctoides* group, comprising of a single species, stump-tailed macaques *M. arctoides*.

Delson (1980) subsequently modified this classification by placing stump-tailed macaques within the *sinica* species-group and by removing the Barbary macaque from the *silenus* species-group to make it a sister taxon to all the Asian species-groups. Later, Groves (2001) divided the genus into six species-groups by granting Sulawesi macaques their own group and separating the *mulatta* group into a monophyletic one. The latest classification by Wilson and Reeder (2005), however, suggests the recognition of five species-groups:

1. the *sylvanus* group (monotypic);
2. the *nemestrina* group (*M. silenus*, *M. leonina*, *M. nemestrina*, *M. pogensis*, *M. siberu*, *M. maura*, *M. ochrata*, *M. tonkeana*, *M. becki*, *M. nigrescens* and *M. nigra*);
3. the *fascicularis* group (*M. fascicularis* and *M. arctoides*);
4. the mulatta group (M. mulatta, M. cyclopis and M. fuscata);
5. the sinica group (M. sinica, M. radiata, M. assamensis and M. thibetana).

Despite the discrepancy in the species-group definitions, a consensus has been reached on the existence of at least three species-groups, the silenus group, the sinica group and the fascicularis group (Delson 1980; Fooden and Lanyon 1989; Hayasaka et al. 1996). The taxonomic positions of Barbary macaques, stump-tailed macaques and Sulawesi macaques are still debated as to whether they belong to a monophyletic group that includes other extant macaques, or they should be classified in their own group as a sister to all the other macaques.

Previously, macaque systematics and evolution were largely based on only morphological traits, such as the male and female reproductive organs (Fooden 1976; Fooden 1980; Fooden and Lanyon 1989), dentition or the structure of the cranium (Delson 1980). In the last thirty years, however, molecular data in the form of allozymes (Melnick and Kidd 1985; Fa 1989), mitochondrial DNA (mtDNA) sequences (Zhang and Shi 1993; Li and Zhang 2005; Smith et al. 2007) and nuclear DNA markers (Deinard and Smith 2001; Tosi et al. 2002, 2003) have gradually taken over a more important role in reconstructing macaque phylogeny and resolving taxonomic contentions. The phylogenetic relationships among the different species and species-groups have, nevertheless, not been entirely resolved. For example, the phylogenetic relationship among the Asian species-groups has always been problematic because of their oft-estimated paraphyletic relationships, which have yielded variable results (Delson 1980; Morales and Melnick 1998; Tosi et al. 2000, 2003). It has been suggested that such complexity has stemmed from the incipient speciation of many of the taxa, coupled with many historical instances of hybridisation and introgressions (Tosi et al. 2003). It is possible that given the recent origin of the genus, many “species” are still far from being monophyletic and are effectively composed of “species complexes”, which still lack any considerable reproductive barrier within them in spite of containing members that differ extensively in their morphometric features.

### 1.5.2 Macaque phylogeography

Very few studies exist on the phylogeography of macaques. In one of the earliest attempts, Hoelzer et al. (1994) found support for female philopatry and male migration in toque macaques in Sri Lanka. They discovered that even at a very small scale, the species showed divergent mtDNA haplotypes that were spatially segregated. More recently, two macaque species, the
Japanese macaque and the Barbary macaque, have been studied in fair detail. Marmi et al. (2004) reported extensive genetic similarities between rhesus and Japanese macaques based on an approximately 400bp-long mitochondrial control region sequence. On the basis of the demographic analyses of the genetic data, they further estimated the probable time of colonisation of Japan by the ancestors of the Japanese macaque between 0.31 and 0.88 mya. This female-bonded species also exhibited strong geographical differentiation of its populations, as could be surmised from maternally inherited mtDNA data, suggesting female philopatry, a typical feature of macaque biology (Marmi et al. 2004). Later, in a more detailed study, Kawamoto et al. (2007) discovered the presence of two putative major mtDNA haplogroups in the species, one centred in eastern and the other in western Japan. They further concluded that the direction of colonisation was probably from west to east and that the populations showed signature of expansion following the LGM (Kawamoto et al. 2007). Consequently, this study, for the first time, highlighted the considerable impact Pleistocene glaciations have had on a temperate primate species. In a follow-up exploration, Kawamoto et al. (2008) investigated genetic variation in a specific population of the species in the Shimokita peninsula, which represents the northernmost distribution limit of the Japanese macaque. Interestingly, the study population was completely isolated from the other populations of the species. Using both autosomal, hence biparentally inherited, microsatellite loci and paternally inherited Y-chromosomal microsatellite loci, they detected a weak signature of a population bottleneck in the species. Consequently, Kawamoto and colleagues (2008) speculated that an ancient climate-driven bottleneck must have occurred during the warm inter-glacial periods but could not rule out more recent impacts of severe hunting of the species by humans.

At the other end of the macaque distribution range, Modolo and colleagues (2005) studied the phylogeography of the only African macaque, the Barbary macaque, using the mitochondrial hypervariable segment 1. Once distributed throughout north Africa, the species is now threatened by severe habitat fragmentation and is consequently restricted to isolated forest fragments in the northern fringes of Algeria and Morocco. The phylogeographic analysis clearly indicated high levels of population differentiation with possible alternating cycles of population-size changes (Modolo et al. 2005). The authors suggested that both, the usual pattern of cercopithecine female philopatry and the long-term fragmentation of the habitat by anthropogenic activities may have been the factors driving population differentiation. In addition, it was postulated that the Pleistocene climatic fluctuations might have also impacted the historical population size of the species (Modolo et al. 2005).
Among other macaque species, Smith and McDonough (2005) reported the presence of considerable population differentiation in the rhesus macaque. They observed that Indian and Chinese rhesus macaques were probably isolated during most of the Pleistocene, during which time Indian rhesus macaques also experienced a severe population bottleneck. Subsequently, however, restoration of some gene flow westward into India was believed to have occurred, according to the mtDNA results obtained (Smith and McDonough 2005). Later, a genome-wide study of the rhesus macaque populations estimated the separation of the two populations to have occurred approximately during the early- to mid-Pleistocene (Hernandez et al. 2007).

Overall, studies of macaque phylogeography agree to a sharp geographical clustering of different species (Melnick and Hoelzer 1992; Evans et al. 2001; Modolo et al. 2005; Smith and McDonough 2005; Kawamoto et al. 2008) and to limited mtDNA variation within certain populations (Melnick and Hoelzer 1992; Hoelzer et al. 1994; Perwitasari-Farajallah et al. 2001; Modolo et al. 2005; Smith and McDonough 2005). Such a general pattern could stem both, from the social system of female philopatry in macaques as well as from the long-term geographical isolation of certain specific populations. Using biparentally and maternally inherited markers simultaneous, we can, however, differentiate between these two factors. Moreover, Pleistocene glaciations appear to have significantly influenced the phylogeography of macaques. It is, however, far from clear if this factor was equally influential in shaping the phylogeography of the more tropical species, which have never been investigated. Finally, most of these studies have focussed on species that are either highly threatened or whose habitats are severely fragmented. It is time that we compare the phylogeographic patterns exhibited by such species with that of more continuously distributed species in order to gain a broader perspective on the factors that may have shaped genetic variation across macaque species, in general.

We, therefore, decided to investigate the comparative phylogeography and demographic history of the Arunachal macaque, an ecologically conservative, subtropical species, patchily distributed in the mountainous valleys of the Eastern Himalayas and that of the bonnet macaque, a generalist species widely and continuously distributed across the plains of peninsular India.
1.6 The study species

1.6.1 The Arunachal macaque

The Arunachal macaque (*Macaca munzala*, Sinha, Datta, Madhusudan and Mishra 2005; Fig. 1.2) is a newly described primate species from the Eastern Himalayan mountain ranges in the northeast Indian state of Arunachal Pradesh (Sinha et al. 2005). It is a large-bodied, dark coloured, montane Old World monkey (OWM) which have been classified on the basis of its penile morphology, within the *sinica* species-group of the genus, which also includes the Assamese macaque *M. assamensis*, the Tibetan macaque *M. thibetana* the bonnet macaque *M. radiata* and the toque macaque *M. sinica* (Sinha et al. 2005).

A part of the species’ known distribution range has been explored since its discovery and the species has mostly been seen or reported from altitudes between 2,000 and 3,000 m in the districts of Tawang, West Kameng, Upper Subansiri and West Siang in Arunachal Pradesh while the local people have reported its seasonal occurrence upto 3,500 m (Sinha et al. 2005; Kumar et al. 2008). It is possible, given the wide distribution range of the species that the species may also occur in the adjoin areas of Bhutan and Tibet in the west and the north, although these regions have not yet been explored for the species. The easternmost distribution of the Arunachal macaque, however, is likely to extend only till the river Brahmaputra, a natural barrier that presumably separates this species from populations of the Eastern Assamese macaque *M. assamensis assamensis*.

The Arunachal macaque have largely been sighted in multimale-multifemale troops, with group sizes ranging from five to over 60 individuals, with a mean size of 21.6 (± 10.4) individuals (Sinha et al. 2006a). On the basis of its social organisation, the species appears to be a typical member of the *sinica* species-group of the macaques exhibiting a matrifocal society with tolerant social relationships among the adult males and females within a troop (Sinha et al. 2013). Generally, each troop consists of one to two adult males, several adult females and a variable number of subadults, juveniles and infants of both sexes (Sinha et al. 2013). On the basis of 22 studied troops in the Tawang district of western Arunachal Pradesh, Kumar et al. (2008) reported the overall adult sex ratio in the species to be 52 males per 100 females, the infant-to-adult female ratio, 17 per 100 females, and the juvenile-to-adult female ratio, 110 per 100 female. It was further discovered, based on preliminary demographic data on six troops in the
Zemithang region of the same district, that the adult females in these troops may have an inter-birth interval of two years (Kumar et al. 2008; Sinha et al. 2013).

![Figure 1.2 An adult male Arunachal macaque on a rock façade in the Zemithang region of Tawang district in Arunachal Pradesh. The typical dark body colouration and short tail is visible along with the seasonal light forehead patch typical of the species (from Sinha et al. 2005; photo credit: M D Madhusudan)](image)

The Arunachal macaque has largely been reported from two contrasting landscapes: subtropical forests and areas of intense human cultivation and habitation. The subtropical vegetation mostly includes broadleaved forests (up to 3000 m, with *Rhododendron, Acer, Alnus*, and *Quercus* as the dominant tree species), conifer-broadleaved forests (3000-4200 m, with *Abies densa, Juniperus, Larix, Picea, Rhododendron*, and *Quercus*), and forest clearings, largely pastureland created by clearing and burning broadleaved forests and conifer-broadleaved forests, with shrubs such as *Rosa* and *Berberis*, and forbs such as *Anaphalis, Potentilla, Sambucus, Rumex*, and *Senecio*. At low- to mid-elevations, the broad-leaved forests are also significantly degraded in the vicinity of human
habitations and appear as secondary scrub, with reduced tree cover and dominated by tree species such as *Erythrina*, *Rhus*, *Elaeagnus* and *Debregeasia* (Kumar et al. 2008).

On the basis of recent studies, the species appears to have fairly well delimited home ranges, varying in size from 7 to 55 ha (Kumar et al. 2008; Mendiratta et al. 2009). While the home ranges of neighbouring troops may also overlap to a certain extent (Kumar et al. 2008), with the resident troops usually co-existing peacefully, physical overlaps, nevertheless, are regularly evaded to avoid agonistic inter-group encounters by scheduling non-overlapping daily movement by each troop (Kumar, pers. obs.). Movement within and out of the often loosely defined home range, however, appears to be primarily influenced by availability of food (Mendiratta et al. 2009).

This species is possibly one of the most endangered of all Indian primates (IUCN 2012). The Eastern Himalaya is also home to approximately 145 tribal communities, which have increased markedly in population size in recent years (four-fold in the state of Arunachal Pradesh alone since 1947; Anonymous 2006). The demand for wild meat from urban populations, coupled with the diffusion of new hunting technologies, has also fuelled the rapid extinction of many Himalayan species, including primates (Aiyadurai 2011). The most serious survival threat faced by the species over much of its distribution range in central (Upper Subansiri and West Siang districts) and western (Tawang and West Kameng districts) Arunachal Pradesh today is hunting (Kumar et al. 2007, 2008; N. Gama, unpublished data). Although variable across regions, the most important reasons cited for its extensive killing has been in retaliation to crop damage caused by the simians, for food and sport, for pet- and commercial trade, and for the alleged medicinal value of its flesh for both humans and livestock (Sinha et al. 2006b). It was estimated that an average of about 30 individual macaques were killed per village (12 villages surveyed in the four districts) per year (between 2004 and 2007) – a significant number considering that primatologists have been able to record (from both primary and secondary sources) a population of approximately 1,500 macaques across the four surveyed districts of the state so far (Kumar et al. 2007, 2008; N. Gama, unpublished data). Further, the adult females of the species neither all reproduce at any given time nor give birth every year, suggesting a moderate to low birth rate for the surveyed populations as well (Kumar et al. 2008).
1.6.2 *The bonnet macaque*

The bonnet macaque *Macaca radiata* (Fig. 1.3) is an endemic species of peninsular India and is pervasively distributed across a variety of ecological habitats (Sinha 2001a). It is also one of the better-studied primate species from the Indian subcontinent, with descriptive accounts dating back to as early as 1925 (Pocock 1925; Hartman 1938; Simonds 1965; Sugiyama 1971). Fooden (1981 and 1986) have morphologically divided bonnet macaques into two subspecies, the southern ‘pale-bellied’ bonnet macaque (*Macaca radiata diluta*), distributed in the deep southern region of the peninsula encompassing the states of Tamil Nadu and Kerala, and the northern ‘dark-bellied’ bonnet macaque (*Macaca radiata radiata*), with a more widespread distribution through the states of Kerala, Tamil Nadu, Andhra Pradesh, Karnataka, Maharashtra, Goa and Gujarat, extending up to Surat in the northwest of the peninsula and the rivers Krishna and Godavari in the northeast (Kumar et al. 2011).

The bonnet macaque is usually found to live in multimale-multifemale troops of about 15 to 60 individuals (reviewed in Sinha 2001a). The females, being philopatric like those of many other cercopithecine primates, remain in their natal groups throughout their lives, and during adulthood, form strong, linear dominance hierarchies with daughters occupying dominance ranks below those of their mothers. Juvenile and subadult males have mostly been reported to emigrate from their natal troops, another typical cercopithecine feature, few incidences, however, of males staying back in their natal troops and often attaining a high dominance rank have also been reported in this species (Sinha 2001a; Sinha et al. 2005).

Regarding the social organisation in bonnet macaque, a long-term study by Sinha and his colleagues (Ram et al. 2003; Sinha 2005, 2013; Sinha et al. 2003, 2005; Mukhopadhyay and Sinha 2010; Sinha and Mukhopadhyay 2013) on a large, free-ranging population of bonnet macaques in the Bandipur National Park – Mudumalai Wildlife Sanctuary of southern India, however, made observations that were strikingly different from what was previously known about the species. Sinha and colleagues (2003, 2005), most importantly reported the occurrence of a large proportion (approximately 50%) of unique single-male but multifemale troops (henceforth referred to as ‘unimale troops’) in this population. According to them, the existence of such a social organisation in the species had rarely been reported in earlier studies or had been observed in very low proportions.
Sinha et al. (2005) further described the unimale troops to have a highly female-biased sex-ratio across all age classes with extensive variability in behavioural strategies including widespread female emigration, an example of within-species variation in social structure and behavioural strategies (reviewed in Sinha and Mukhopadhyay 2013).

Across its widespread distribution in Indian peninsula, the bonnet macaque usually occupies two major ecological niches: a variety of forest types and areas of human cultivation and habitation. Sinha (2001a) reviewed the different forest types inhabited by the highly adaptable generalist macaques, which include the montane evergreen rainforests of the Nilgiri hills (sometimes even up to an altitude of about 2100 m; Simonds 1965), low-elevation semi-evergreen forests of coastal Kerala, moist and dry deciduous forests of Karnataka and Tamil Nadu, bamboo forests, and the arid scrub of the central areas of the Deccan plateau. Interestingly, Sinha (2001a) further informed that the monkeys are relatively rare in very wet montane forests at higher altitudes, and
the few troops that have been reported often were seen to move down to lower-elevation, drier deciduous forests regularly. Moreover, they simians are supposed to be extremely rare in the *hoblo* forests and in the adjoining grasslands of the Nilgiri hills of southern India (Sinha 2001a).

Bonnet macaques are, however, much more common in areas of human habitation and cultivation, including villages bordering agricultural fields and plantations, small towns (where they are usually encountered at temples or railway and bus stations) or many times even in the large cities like Chennai and Bangalore (reviewed in Sinha 2001a).

Among one of the earliest estimates, Kurup (1981) suggested that the total population of bonnet macaques in the four south Indian states would be of the order of 1,70,000, with about 81,000 monkeys in Karnataka, 64,000 in Andhra Pradesh, 16,000 in Tamil Nadu and 11,000 in Kerala.

What, nevertheless, remains unknown is the rate of decline of many of these populations on the face of the intensification of agriculture in rural areas and the increasing intolerance towards the species in urban localities in the last 30 years.

### 1.6.3 A comparison of the Arunachal macaque and the bonnet macaque

As both the Arunachal and bonnet macaques belong to the *sinica* species-group, the two species could be expected to share various biological traits by phylogenetic descent. Unfortunately, our knowledge of Arunachal macaque is fragmentary particularly due to its recent discovery, relative rarity and difficulty in access. The differences between the two species, nevertheless, appear to be striking, particularly in their ecology and social behaviour. We summarise the known differences between the species below (Table 1.1).

### 1.7 Motivation and objectives of the thesis

As briefly narrated above, we were motivated to explore the differences in phylogeography between the two phylogenetically closely related species, the Arunachal and bonnet macaques, which nevertheless live in rather different ecological environments. We were also interested to examine their demographic histories, particularly with reference to the impacts that Pleistocene glaciations and related climate changes may have had on them, given, of course, that they occur at different latitudes and altitudes. Finally, we planned to investigate the dispersal patterns of the
Table 1.1 A comparison between the study species. (Source: Sinha 2001a, 2013; Sinha et al. 2013; Sinha and Mukhopadhyay 2013)

<table>
<thead>
<tr>
<th>Traits</th>
<th>Arunachal macaque</th>
<th>Bonnet macaque</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geographic location</td>
<td>Southern edge of the Tibetan Plateau, Eastern Himalaya</td>
<td>Indian peninsula</td>
</tr>
<tr>
<td>Climate</td>
<td>Subtropical</td>
<td>Tropical</td>
</tr>
<tr>
<td>Topography</td>
<td>Montane, interspersed with valleys</td>
<td>Predominantly plain</td>
</tr>
<tr>
<td>Group size</td>
<td>Five – 60 individuals</td>
<td>15 – 60 individuals</td>
</tr>
<tr>
<td>Home range</td>
<td>Seven – 50 ha, seasonally variable</td>
<td>26 ha – 5 km$^2$</td>
</tr>
<tr>
<td>Altitude</td>
<td>2,000 – 3,500 m</td>
<td>Less than 1,000 m, occasionally upto 2,000 m in the Nilgiri Hills</td>
</tr>
<tr>
<td>Habitat types</td>
<td>Subtropical and temperate forests, and areas of human cultivation and habitation; specialist species</td>
<td>Predominantly low-lying, semi-evergreen forests; moist and dry deciduous forests; arid scrub jungles of the Deccan Plateau; sometimes montane evergreen rainforests of the Nilgiri Hills; rarely in wet montane forests, <em>shola</em> forests and in the adjoining grasslands at higher altitudes; areas of human cultivation and habitation; generalist species</td>
</tr>
<tr>
<td>Female philopatry</td>
<td>Present</td>
<td>Predominant</td>
</tr>
<tr>
<td>Male philopatry</td>
<td>Unknown</td>
<td>Occasional</td>
</tr>
<tr>
<td>Female dispersal</td>
<td>Unknown</td>
<td>Present</td>
</tr>
<tr>
<td>Male dispersal</td>
<td>Present</td>
<td>Predominant</td>
</tr>
<tr>
<td>Social organisation</td>
<td>Multimale-multifemale</td>
<td>Predominantly multimale-multifemale</td>
</tr>
<tr>
<td>Unimale groups</td>
<td>Unknown</td>
<td>Present</td>
</tr>
<tr>
<td>Social bonding</td>
<td>Female-bonded, male bonding unknown</td>
<td>Female-bonded, male bonding common</td>
</tr>
<tr>
<td>Allogrooming</td>
<td>Present among females, unknown among males</td>
<td>Present among both sexes</td>
</tr>
<tr>
<td>Threats</td>
<td>Hunting, habitat modification, conflict over crop-raiding</td>
<td>Rapid habitat modification, conflict over crop-raiding</td>
</tr>
<tr>
<td>Status</td>
<td>Endangered</td>
<td>Least concern, but potentially threatened</td>
</tr>
</tbody>
</table>
two female-bonded primate species using both biparentally and maternally inherited genetic markers. We provide below a justification for the two species that we chose to study and list our predictions about their population genetic structure on the basis of their similarities and differences (Table 1.2).

Table 1.2 Our predictions for the population genetic structure of the two study species.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Arunachal macaque</th>
<th>Bonnet macaque</th>
<th>Population genetic structure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Arunachal macaque</td>
</tr>
<tr>
<td>Topography</td>
<td>Mountains and valleys</td>
<td>Plains</td>
<td>High</td>
</tr>
<tr>
<td>Habitat</td>
<td>Sub-tropical</td>
<td>Tropical</td>
<td>Simple pattern</td>
</tr>
<tr>
<td>Ecology</td>
<td>Specialist</td>
<td>Generalist</td>
<td>High</td>
</tr>
<tr>
<td>Pleistocene glaciations</td>
<td>Direct effect</td>
<td>Indirect effect</td>
<td>High</td>
</tr>
<tr>
<td>Sex-biased dispersal</td>
<td>Female philopatry</td>
<td>Female philopatry</td>
<td>High for mtDNA and low for nuclear DNA</td>
</tr>
</tbody>
</table>

1.7.1 Subtropical/temperate versus tropical species

Phylogeography is a young field of evolutionary biology and earth sciences. In spite of its recent conception, we have seen a tremendous development in the field, particularly over the last ten years buoyed by the advancement of molecular tools and computational technologies. We now know significantly more about the relationships between populations and their geography, and how environmental factors such as climate change may have shaped patterns of population distribution. While the picture is much clearer now for temperate habitats and species in Europe
and North America, where the majority of the studies have taken place, less is known about species from the tropics. Tropical ecosystems also harbour a greater diversity of complex interactions than do temperate ecosystems, where Pleistocene glaciations overwhelmingly influenced the phylogeography of organisms, including both plants and animals.

Tropical habitats, in addition, comprise highly variable ecosystems that harbour a great variety of life forms. Furthermore, there exists a large region where temperate climate changes into a tropical one, gradually creating a complex gradient of habitats, which also remain unstudied. Having understood temperate phylogeography to a certain degree, it is necessary that we now perform complimentary studies on the species from the tropics and the transition zone so that we can discover the processes and patterns that may explain the phylogeography and evolutionary history of these taxa.

The Indian subcontinent possesses both the tropical and the transition habitats and yet, unfortunately, boasts of only a few phylogeographic studies to date. Consequently, we chose to focus on two regions in the Indian subcontinent. We chose the Indian peninsula as a typical tropical habitat and Arunachal Pradesh, which falls within the mountainous southern edge of Tibetan plateau in addition to being part of the Eastern Himalaya biodiversity hotspot, as a representative of the transition zone. There have been very few phylogeographic studies reported from both these regions, which, in spite of being biodiversity rich, seem poised to lose most of their habitats and species due to high levels of anthropogenic activities. Hence, these regions have become important hotspots for biodiversity conservation in recent years.

1.7.2 Generalist versus specialist species

The phylogeography of apes such as the chimpanzee, gorilla and the orang-utan are well studied in comparison to groups like Old World monkeys which occupy a much larger geographical ranges with highly variable habitats. The reason for a relatively greater emphasis on the apes is partly because they are charismatic species. Most of the ape species are also highly threatened due to poaching and habitat degradation. Apes are more specialised in their habitat requirements and hence, more vulnerable to such changes. On the other hand, macaques, prime representatives of Old World monkeys, are highly adaptable but, ironically, this adaptability may have ensured that they remain academically neglected. Due to their vulnerability, therefore, ecologically specialised species had been studied more zealously while generalist species like
macaques were largely ignored. However, to understand how primates cope with long-term changes in large-scale environmental factors, we need to study both the specialist and the generalist species. A comparison between these two kinds of species promises to open up new perspectives on the nature of the adaptability shown by certain taxa and allows us to develop viable management strategies for them. Consequently, we chose to compare the Arunachal macaque, the montane and specialist species from Arunachal Pradesh, with the bonnet macaque, a generalist “weed” species from the plains of the Indian peninsula.

1.8 The structure of the thesis

At a very basic level, this thesis attempts to compare the phylogeography of two contrasting primate species to gain insights into the evolutionary processes that have shaped their populations in general. More specifically, there are two aspects to the thesis. First, in a descriptive endeavour, we lay down the differences in spatial and temporal distribution of genetic variation in this pair of complementary primate species. Second, we identify some of the factors, biotic and abiotic, that may have been responsible for the observed distribution of the genetic variation.

In practice, the thesis consists of four chapters, excluding this introduction. In Chapter 2, we reconstruct the phylogeny of the of the *sinica* species-group including the Arunachal macaque and the bonnet macaque. We also trace the evolutionary history of the two species using molecular dating. This chapter has already been published as a scientific paper in the peer-reviewed journal, Molecular Phylogenetics and Evolution.

In Chapter 3, we first describe the population genetic structures of the two study species, the Arunachal macaque and the bonnet macaque, using mtDNA and nuclear microsatellite loci. We then compare their population structure to identify the potential factors that may have shaped them; these include Pleistocene climatic fluctuations, anthropogenic factors and the socio-behaviour of the two species.

In Chapter 4, we trace the demographic histories of the two species, and use mtDNA and microsatellite loci to identify changes in their population size during the Pleistocene as well as in
the more recent Holocene periods. We then attempt to identify the potential environmental factors that may, in retrospect, have wrought changes in their demographic structure in the past.

Finally, in Chapter 5, we synthesise all our findings to first describe the marked differences between the two study species and then outline their general importance for our understanding of primate phylogeography. We then describe the general differences between our results and those that have been obtained from comparable studies in temperate regions. We conclude our chapter by commenting on the implications of our study for mammalian phylogeography and for biodiversity conservation, in general.

1.9 References


