Chapter 1: General introduction

1.1 Investigation of genetic variation

Genetically, organisms are structured in the hierarchy of genes, genotypes, populations and species. The population genetic structure is the most fundamental piece of information for a species that requires genetic management (Brown, 1978, Yeh, 2000). To date, the description of population genetic structure and its dynamics has been based on allele and genotype frequencies in sampled populations with simply inherited traits whose transmissions follow Mendelian rules (Yeh, 2000). The assessment of genetic variation involves the determination of genetic multiplicity (i.e. proportion of polymorphic loci, number of alleles per locus and allelic richness) and genetic diversity (i.e. effective number of alleles, expected and observed heterozygosity) (Finkeldey and Hattemer, 2007). Its pattern is the result of the impact of evolutionary factors, which are mutation, gene flow and migration, mating system, genetic drift and selection (Wright, 1931; Finkeldey and Hattemer, 2007). The Hardy-Weinberg Principle, which states that in a large random-mating population with non-overlapping generations, the allele and genotype frequencies will remain constant from generation to generation when there is no mutation, migration and natural selection provides the foundation for all population genetic investigation (Yeh, 2000).

In plants, two very distinct vehicles mediate the dispersal function: the male gametophyte (pollen) and the young sporophyte (seed) (Bensch and Åkesson, 2005). Hence, in populations continuously distributed over a larger area, genetic isolation by distance is expected, since it is assumed that gene flow happens mainly between nearby locations (Rousset, 1977).

Genetic variation within and among populations can be investigated by employing biochemical markers (isozymes/allozymes), direct DNA sequencing and using molecular (DNA) markers (Weising *et al*., 2005). Until recently, research on the genetics of tropical trees was confined largely to allozyme studies of the genetic structure of adults in continuous forests (Hamrick and Murawski, 1991; Loveless, 1992). The first DNA marker exploited is referred to as Restriction Fragment Length Polymorphism (RFLPs; Botstein *et*

al., 1980) and involves the analysis of restriction-digested DNA in the so-called Southern blotting technique and hybridization with a sequence-specific probe (Weising *et al*., 2005). The recent molecular techniques such as Random Amplified Polymorphic DNA (RAPD; Williams *et al*., 1990), Intersimple Sequence Repeat Polymorphism (ISSR; Wolfe and Liston, 1998), microsatellites (also known as Simple Sequence Repeats, SSRs; Akkaya *et al*., 1992), Amplified Fragment Length Polymorphism (AFLP; Vos *et al*., 1995) as well as inverse sequence-tagged repeat (ISTR; Rohde, 1996) involve the Polymerase Chain Reaction (PCR), in which amplification of the fragments of genomic DNA is conducted using a heat-resistant DNA polymerase (Taq polymerase), primers and deoxyribonucleotide triphosphates at high temperatures (Saiki *et al*., 1988). The use of molecular markers in the investigation of genetic variation is getting a wide acceptance and broad application in fields such as phylogeny, taxonomy, ecology and genetics and breeding (Weising *et al*., 2005).

The DNA under investigation can be nuclear, extra-nuclear (organelle DNA) or the whole genome depending on the type of marker employed. The organelle and nuclear genes are inherited uniparentally and biparentally, respectively, and can be used to study the distribution of genetic diversity within and among populations and to infer the relative importance of seed and pollen dispersal (Petit *et al*., 1993, 2005). The nuclear DNA (nDNA) is subjected to intergenerational recombination whereas organelle genomes can exhibit intragenerational segregation (Murrey *et al*., 2000).

1.2 Physiography, climate and vegetation of Ethiopia

Ethiopia is located in the north-eastern part of Africa, popularly known as the Horn, between $3^0$24' and $14^0$53' N, and $32^0$42' and $48^0$12'E, and covers a total area of 1.13 million km^2 (CSA, 2001). It is bordered in the north and north-east by Eritrea, in the east by Djibouti and Somalia, in the south by Kenya, and in the west and southwest by Sudan. The population is estimated at more than 75 million, with 85% depending on agriculture, which is the mainstay of Ethiopian economy accounting for 54% of the GDP and about 90% of the exports (CSA, 2001).

The physiographic features include rugged mountains, deep gorges and river valleys, and rolling plains (Bekele, 1994). The very large dissected, dome-shaped mountain massif

consists of two plateaus, which are referred to as the North-Western (NW) Highlands and the South-Eastern (SE) Highlands (highlands being areas above 1500 m above sea level). Generally, the Ethiopian highlands are the biggest in tropical Africa (Friis *et al*., 2001). The altitude in Ethiopia ranges from 120 m below sea level at the Afar depression to 4370 m on Tullu Demtu (the highest peak in the SE) and 4620 m on Mount Ras-Dashen (the highest peak in the NW). The NW highlands are subdivided into a northern and a southern part by the extensive and up to 1000 m deep and 10-50 km wide Blue Nile (Abbay) Valley. The northern part is highly dissected, but with altitudes between ca. 2000 m and ca. 3000 m, they are generally higher than the southern highlands, which are less dissected and have extensive areas between ca. 1500 m and ca. 2000 m (Friis *et al*., 2001). Another classification has recognized three subunits in the NW highlands; namely, the north, central and the southwest (SW) (Friis, 1992). The geologically active Great Rift Valley extends from Lake Turkana in the SE to the Afar region in the north-east (NE) dissecting the two highland massifs in the Lake Region. In addition, lowlands, steppes and semi-desert areas (areas less than 1500 m in altitude mainly the Western Lowlands, Borana and Ogaden) stretch from the respective slopes and bound each of the massifs. This great terrain diversity is responsible for wide variations in climate, soil and natural vegetation (Friis, 2001).

The climate of Ethiopia is tropical monsoon with wide topographic-induced variations. The SW Highlands receive the highest annual rainfall (1400-2200 mm per annum) and the arid lowlands in Afar and in the SE Lowlands receive as little as 100 mm to 300 mm. The monthly average temperature shows very little seasonal variation, 2^{o}C in the south and 6^{o}C in the north, and the mean annual temperature decreases with altitude (Friis 1992). Generally, low temperatures are attained during the rainy months and high temperatures occur during the dry and sunny season. The climate of Ethiopia in terms of temperature variations can be broadly classified into three types (Breitenbach, 1963): Cool Zone (Dega)- cold mountains above 2400-2600 m, with average temperatures between 10 and 16°C; Temperate Zone (Woina-Dega)- temperate highlands between 1600-1900 m to 2400-2600 m, with mean temperatures between 16 to 20°C; and Hot Zone (Kolla)- hot lowlands below 1600-1900 m, with both tropical and arid conditions, and with mean temperatures between 20 and 29°C. A more complex climatic classification identifies three zones (Dry, Tropical and Temperate) with three subdivisions each (Anonymous, 1992).

The vegetation of Ethiopia has been surveyed quite extensively (e.g. von Breitenbach, 1963; Greenway 1973; Chaffey, 1979). The types and geographic distributions of the vegetation based on Friis (1992) can be summarized as follows: Deciduous woodland and wooded grassland cover the western lowlands, with the floristic composition varying considerably from the north to the south. The undifferentiated woodland, which is composed of species such as *Combretum collinum* and *Terminalia brownie*, is found on the escarpment of the NW highlands in the altitudinal range from 500 to 1500 m. Transitional rain forests exist on the SW slopes of the NW highlands. The Afromontane rain forests are located at the elevations between 1500 and 2000 m in the SW part of the NW highlands and in some parts of the SE highlands. Under drier conditions and at higher elevations, the forests in both plateaus tend to be dominated by the conifers *Podocarpus falcatus* and *Juniperus procera*, which are classified as undifferentiated Afromontane forests. The Rift Valley is mainly covered by *Acacia* woodland in the lakes area and with desert scrubland in the Afar area. The western lowlands are humid and are mainly characterized by a single semi-deciduous forest (Guineo-Congolian forest) and extensive broadleaved woodland. The SE lowlands are dry lowlands with *Acacia* and other semi-desert woody species.

A recent national document (IBC, 2005) identifies eight natural ecosystems in the country (1) Afroalpine and subafroalpine ecosystems (areas above 3200 m), (2) Dry evergreen montane forest and grassland complexes (an extensive area in both plateaus between [1600-] 1900-3300 m), (3) Moist evergreen montane forest ecosystems (situated in the SW and SE between 1500 and 2600m), (4) Lowland semi-evergreen forest ecosystems (found between 450 and 650 m), (5) *Acacia-commiphora* woodland ecosystems (occurs between 900 and 1900 m in the Rift valley and SE lowlands), (6) *Combretum-Terminalia* woodland ecosystems (found in the western escarpments between 500 and 1900 m), (7) Desert and semi-desert scrubland ecosystems (found in areas below 500 m), and (8) Aquatic ecosystems, which includes wetlands and lake areas.

However, the natural ecosystems and the natural vegetation have been greatly altered over the years. For example, the large areas of the forests over both the NW and the SE highlands have been replaced by secondary evergreen bush land, wooded grassland or farmland. The forests (not including the woodland and other vegetation cover) were reduced from the original 35% to 16% in 1952 (Sayer *et al.*, 1992) and to 3.6% currently (Anonymous, 2004). Remnants are left on the less accessible areas mainly in the SW and

SE. Isolated forest trees occur scattered throughout most of the highlands (Figure 1), suggesting the former presence of forest there. Patches of forest and woodland, with a species composition similar to that of the remaining natural forests, are very common around churchyards and graveyards, which are abundant in the highlands (Bekele, 1994). Deforestation has its longest history in the northern part of the country due to the farming activities for several centuries practiced there.

Figure 1 Contrasting view of continuous and scattered populations

1.3 *Cordia africana* Lam.

1.3.1 Taxonomy and botanic description

Cordia L. (generic name after Valeris Cordus, a German botanist) is a pantropical genus of about 250 species belonging to Boraginaceae Juss. (ICRAF, 1998), a plant family comprising about 100 genera and 2000 species that are characterized by flowers in helicoid cymes and by coarsely hairy herbage (Carr, 2006). *Cordia africana* Lam. (Synonym: *Cordia abyssinica* R. Br.) is a tree (rarely shrubby) species. Its English common names are East African cordia or large-leafed cordia or Sudan teak (ICRAF, 1998). On average, it attains a height between 14 and 21 m and a diameter at breast height (dbh) between 0.60 and 0.90 cm (chapter 5), and shows great morphological variation (Figure 2).

The botanical description of *Cordia africana* (Warfa 1988) is as follows: Crown spreading, umbrella-shaped or rounded. Bole typically curved or crooked. Bark greyish-brown to dark brown, smooth in young trees, but soon becoming rough and longitudinally fissured with

age; young branchlets with sparse long hairs. Leaves alternate, simple, ovate to subcircular, 7.5-17.5 (max. 30) cm long, 3.5-10.2 (max. 30) cm broad; thinly leathery; dark green above, paler green and velvety below, with prominent parallel tertiary net-nerves (about 7 pairs of lateral nerves); apex broadly tapering or rounded; base rounded to shallowly lobed; margin entire; petiole slender, 2.5-7.6 cm long. Buds oval, stalkless, pleated open into flowers that are bisexual, white, sweet scented, shortly pedicelate or subsessile, massed in compact panicles covering the crown, with a white mass of attractive flowers; calyx less than 1 cm long, strongly ribbed, back of lobes covered with short, soft, brown hairs; corolla lobes crinkled, white, long-exerted, funnel-shaped, about 2.5 cm long; cymes with many flowers. Fruit a drupe, smooth, spherical, oval tipped, fleshy, 1.3-1.5 cm long; green when young, yellow to orange when mature; with a sweet, mucilaginous pulp and short remains of the calyx at the base; contains 2-4 seeds, which lack endosperm.

Figure 2 Morphological variations and features in *Cordia africana*

1.3.2 Natural distribution and habitat

Cordia africana is native to Angola, the Democratic Republic of Congo, Djibouti, Eritrea, Ethiopia, Ghana, Guinea, Kenya, Malawi, Mozambique, South Africa, Sudan, Tanzania, Uganda, Zimbabwe, Saudi Arabia and Yemen (Warfa, 1988; Friis 1992). In Ethiopia, it is widespread in Broadleaved Afromontane Rain Forests, Undifferentiated (Dry) Afromontane Forests ('mixed *Podocarpus* forest') and in riverine forests as well as in the western lowlands (Friis, 1992; Figure 3). Generally, the species grows in areas with altitudes between 550 and 2600 m a.s.l. and annual rainfall of 700 to 2000 mm (Friis, 1992). It is an early colonizer in forest re-growth and is often found along forest margins, regenerates in clearings and forest gaps (Fichtl and Admasu, 1994; Derero *et al.*, 2003). It has a light quality sensing mechanism that hinders it from germinating beneath leaves (Yirdaw and Leinonen, 2002).

The natural distribution, the habitats and the populations of the species are very much affected, like other forest tree species, by deforestation, fragmentation and selective logging. Especially the northern part of the country represents an extreme case of deforestation in which the species is represented by scattered trees on farmlands, in and around homesteads, church compounds and graveyards; except at some spots, such as Zeghie Peninsula, where a relatively continuous forest exists.

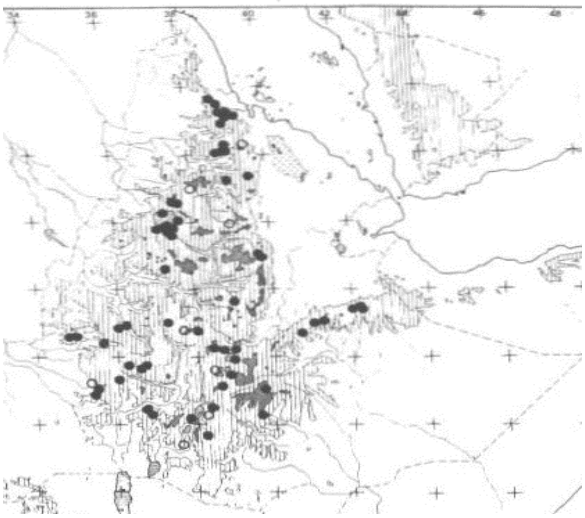

Figure 3 Natural distribution of *Cordia africana* in Ethiopia and Eritrea (from Friis, 1992).