REPRODUCTIVE BIOLOGY AND POPULATION ECOLOGY OF *Senegalia senegal* (L.) BRITTON WITHIN LAKE BARINGO WOODLAND ECOSYSTEM, KENYA

By

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DECLARATION

CANDIDATE

I hereby declare that this thesis is entirely my original work and has not been submitted for a degree award in any other University.

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DEDICATION

I dedicate this work to my wife Eunice and children Adrian, Xavier and Victoria in appreciation of their sacrifices, patience and support during the study. Their support gave me the much needed strength and encouragement to accomplish this study.

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LIST OF ABBREVIATIONS AND ACRONAMES

ABI:	Applied Biosystems Inc
AFLP:	Amplified Fragment Length Polymorphism
AMOVA:	Analysis of Molecular Variance
ANOVA:	Analysis of Variance
ASAL:	Arid and Semi Arid Land
CIA:	Chlorophorm: Isoamyl Alcohol
cpDNA:	Chloroplast Dinucleotide Nucleic Acid
CTAB:	Cetyl Trimethyl Ammonium Bromide
DBH:	Diameter at Breast Height
EDTA:	Ethylene Diamine Tetraacetic Acid
ESTP:	Expressed Sequence Tag Polymorphism
EST-SSR:	Expresed Tag Simple Sequence Repeart
FAO:	Food and Agricultural Organizations of the United Nations
GOK:	Government of Kenya
HCl:	Hydrochloric Acid
HWE:	Hardy Weinberg Equilibrium
IAM:	Infinite Alleles Model
IBPGR:	International Board for Plant Genetic Resources
ICRAF:	International Centre for Research in Agroforestry
ISSR:	Inter Simple Sequence Repeat
LSD:	Least Significant Difference
mtDNA:	Mitochondrial dinucleotide nucleic acid
NaCl:	Sodium Chloride

NAS:	National Academy of Science
OLS:	Ordinary Least Significance
PCoA:	Principal Coordinate Analysis
PCR:	Polymerase Chain Reaction
PIC:	Polymorphic Information Content
PWC:	Price Waterhouse Coopers
RAPD:	Random Amplified Polymorphic Dinucleotide Nucleic Acid
RFLP:	Restricted Fragment Length Polymorphism
SCD:	Size Class Distribution
SD:	Standard Deviation
SNP:	Single Nucleotide Polymorphism
SSCP:	Single Strand Conformation Polymorphism
SSR:	Simple Sequence Repeat
TE:	Tris EDTA
TPM:	Two Phase Mutation
UNEP:	The United Nations Environmental Program
UNESCO:	The United Nations Educational, Scientific and Cultural Organization
UPGMA:	Unweighted Pair Group Method with Arithmetic mean
USA:	United States of America
UV:	Ultra Violet
KEFRI:	Kenya Forestry Research Institute
NSERC:	Natural Science and Engineering Research Council
IFS:	International Foundation for Science

ABSTRACT

Senegalia senegal (L.) Britton is an economically important dryland tree species known for gum arabic production and soil fertility enhancement. However, the species is currently under considerable ecological threat due to overexploitation and habitat degradation. The present investigation was designed to determine the effects of anthropogenic disturbances on reproduction and population structure of *S. senegal* within Lake Baringo woodland ecosystem. The study assessed impacts of anthropogenic disturbance on genetic diversity, natural regeneration and population structure, phenological events, mating systems and gene flow patterns of *S. senegal*. Eleven natural populations of *S. senegal* within Lake Baringo woodland ecosystem were sampled for genetic diversity study. The populations were grouped into two disturbance levels based on population disturbance index (PDI). Within each population, 30 trees were sampled from which fresh leaf tissues were collected. Data on natural regeneration and population structure was collected from 60 plots measuring 20 x 20 m systematically distributed within four natural populations. *Senegalia senegal* trees within these plots were measured for height and diameter at breast height (dbh) and categorized as seedlings, saplings or adult trees. Thirty adult trees in the four populations were assessed at a fortnight interval for a period of 24 months for phenological patterns. For mating systems study, seeds collected from 20 adult tree from two populations with contrasting disturbance levels were sampled. All *S. senegal* adult trees and seedlings from the two populations studied for mating systems were sampled for geneflow analysis. Twelve nuclear microsatellite markers previously developed for *S. senegal* were used to investigate the mating patterns and gene flow of the species within the ecosystem. The markers were also used to determine the impact of human disturbance on the species genetic diversity. Higher seedling (1778±452) and sapling (1294±274) densities were observed in lightly disturbed population than in heavily disturbed populations (seedling, 1206±235; sapling, 717±197). Lightly disturbed populations revealed a ‘reversed J-shape’ size-class distribution (SCD) indicative of normal and stable population structure. The leafing, flowering and fruiting pattern of *S. senegal* were bimodal and followed the rainfall patterns within the ecosystem. Significant variations in floral and fruit morphology were evident. Lightly disturbed populations had higher gene diversity ($H_E = 0.635$), mean number of alleles ($N_A = 7.31$) and allelic richness ($A_R = 6.72$) than heavily disturbed populations ($H_E = 0.577$; $N_A = 7.02$; $A_R = 6.56$). The species displayed a predominantly outcrossing mating pattern. However, heavily disturbed population showed significantly lower outcrossing rates ($tm = 0.833$; $p < 0.05$) than the lightly disturbed population ($tm = 1.000$; $p > 0.05$). Multilocus analysis showed that biparental inbreeding and correlated mating events occurred in the heavily disturbed populations. This suggests that human disturbances may be promoting establishment of seeds produced through consanguineous mating. This suggestion is supported by the estimate of correlation of paternity ($rp = 0.329$) and the mean coefficient of co-ancestrality within families ($\Theta = 0.233$), which indicated that some of the matings in the heavily disturbed populations led to about 23 % of the offspring being full-sibs. Long-distance pollination events and seed dispersal occurred within the woodland. The average pollen dispersal distance ranged from 110 to 400 m. Eventhough mating among near neigbors was common within the populations, the small population sizes were mitigated by the occurance of long distance gene flow through pollen from outside the populations. The results of the present study suggest that *S. senegal* is ecologically and genetically vulnerable to human disturbances and there is need for in-situ conservation strategies to protect the species from further genetic degradation.

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background information

Tropical dry forest and woodland ecosystems are estimated to cover about 40 % of tropical ecosystems globally and about 54 % of the African land cover (Muturi, 2012; Mayaux *et al*., 2005). In Kenya, dryland ecosystems occupy about 87 % of the landmass and sustain more than 30 % of human and 70 % of livestock populations (Muturi, 2012). Characteristically, the dry forests and woodlands are found in the regions receiving between 100-800 mm annual precipitation and experiences dry seasons of about 4-7 months (Bullock *et al*., 1995). The harsh environmental conditions have made the tropical dry forest and woodlands to be very fragile and most tree species found in these ecosystems are at risk of extinction due to disturbances. Recent studies show that dry forests and woodlands are rapidly declining and about 90 % of the remaining dry tropical forests are threatened by further fragmentation and degradation (Yates *et al*., 2000; Miles *et al*., 2006). Despite the harsh climate and human disturbances, the tropical dry forests and woodlands still remains to be habitats for many important tree species (high biodiversity) some of which supply raw materials for industrial use as well as ecosystem services (Chikamai & Odera, 2002; Muturi, 2012). The high level of species diversity in these ecosystems can be linked to the heterogeneous edaphic conditions (Bullock *et al*., 1995).

Lake Baringo woodland, one of such tropical dryland ecosystems, is an important habitat for many plant species (Lelon, 2008). This ecosystem is also known to contribute immensely to the integrity of both Lake Baringo and Bogoria and forms a source of livelihood to many human populations in the basin (GOK, 2009). The Economic mainstay of people living within this ecosystem is livestock production with minor subsistence farming along the seasonal rivers (Lelon, 2008). Though livestock keeping is the economic mainstay in the basin, it is unsustainable and frequently suffers from prolonged droughts (GOK, 2009). Due to lack of alternative sources of livelihoods, the ecosystem is characterized by rampant poverty, food insecurity and overexploitation of forest genetic resources (Lelon, 2008; GOK, 2009). The government of Kenya through vision 2030 blueprint, projects to achieve food security through sustainable management of the drylands and its genetic resources (GOK, 2007). This would partly be achieved through value addition of non-wood forest products which are abundant in these environments. Lake Baringo ecosystem is endowed with such products and many important perennial tree species that have great socio-economic potential (Lelon, 2008).

Senegalia senegal is a high value tree species found in Lake Baringo woodland ecosystem. Commonly known as the ‘gum arabic’ tree, *S. senegal*, have recently caught attention of both local and international stakeholders due to its great socio-economic potential (Fagg & Allison, 2004; Lelon, 2008; Omondi *et al*., 2010b). The species is distributed widely in the dry forests and woodland ecosystems of Kenya. Its distribution ranges from coastal strips to the northern parts of the country with studies showing evidence of commercial exploitation in the entire distribution range (Chikamai & Odera, 2002). However, other socio-economic activities such as human settlements, charcoal production, urban and infrastructural development are also common in most places where the species is found, subjecting the species to great threat of anthropogenic disturbances (Baringo County Government; BCG, 2014).

1.2 Problem statement and justification

Lake Baringo woodland ecosystem, like many other sub-Saharan Africa's vegetation types, is in a state of permanent flux both spatially and temporally (Kiage *et al*., 2007). This has brought negative effect on seed production and natural regeneration capacity of important tree species within the ecosystem. *Senegalia senegal* is one of the species within the woodland ecosystem whose natural regeneration has been negatively affected and does not show normal population structure (Rao *et al*., 1990). In addition, the species flowers profusely but little viable seeds are produced. Cut stumps of the species have also been observed within the ecosystem (Pers. Com). Such phenomenon threatens the persistence and evolutionary potential of the species against the ever changing climate (Kiage *et al*., 2007). The skewed natural regeneration and population structure is suspected to be exacerbated by anthropogenic activities such as charcoal production, electric power production through biomass and livestock overgrazing which are common activities within the ecosystem (Lelon, 2008). Earlier survey reveals that, through human disturbances, most reproductively mature trees within the woodland have been depleted (Lelon, 2008). Such extractions may distort the species biological processes such as reproduction and population dynamics (Dick *et al*., 2008). Furthermore, disturbance of local populations and demographic erosion may intensify genetic segregation and decreases native population in a way which may jeopardise stability of remnant individuals/populations (Ellstrand & Elam, 1993). This therefore calls for development of reliable and effective conservation and sustainable utilization strategies.

To achieve sustainable management of *S. senegal*, there is need for understanding the present status of the species in terms of its genetic diversity, population structure and reproductive potential against the backdrop of the anthropogenic disturbances. Past impacts can be estimated with reference to expert knowledge, but hard data are often lacking more so in the developing world.

Baselines data on impacts of anthropogenic disturbances on vegetation dynamics are therefore needed to quantify future impacts and provide reliable information for conservation purposes.

1.3 Research hypothesis

Reproductive biology and population ecology of *S. senegal* within Lake Baringo woodland ecosystem is significantly affected by anthropogenic disturbances.

1.4 Main objective

This study was undertaken to assess reproductive biology and population ecology of *S. senegal* tree and determine the effects of human disturbances on population structure of the species within Lake Baringo woodland ecosystem.

1.4.1 Specific objectives

i). To assess the genetic variation within and among populations of *S. senegal* within Lake Baringo woodland ecosystem in relation to anthropogenic disturbances.

ii). To determine the effect of anthropogenic disturbances on population structure and natural regeneration patterns of *S. senegal* within Lake Baringo ecosystem.

iii). To determine the diversity of leafing, flowering and fruiting phenology of *S. senegal* among populations within Lake Baringo ecosystem.

iv). To investigate the mating systems of *S. senegal* and determine how anthropogenic disturbances influences these patterns within Lake Baringo woodland ecosystem.

v). To determine the impact of anthropogenic disturbances on gene flow patterns in *S. senegal* within Lake Baringo woodland ecosystem.

1.5 Relevance of the study

Through study of natural regeneration, population structure and genetic diversity, the findings will be usefull in identifying populations within the ecosystem that are negatively affected by anthropogenic disturbances and are at high risk of severe degradation and require immediate conservation intervention. This will be usefull in planning and execution of conservation strategies. Determining the impacts of anthropogenic disturbances on mating systems and gene flow patterns of *S. senegal* and the phenological patterns within the ecosystem will provide insight on the timing and how seed collections for rehabilitation and improvement programs can be structured. This will also be useful in identifying remanat populations or individual trees that may act as linkages among populations for genetic connectivities.

1.6 Thesis structure

This thesis is structured into nine chapters. The current chapter, Chapter one, provides the general introduction of the study and Chapter two addresses the general literature review of the study while Chapter three addresses the general materials and methods. The other five chapers are presented in form of manuscripts prepared for submission to referred journals for publication; Chapter four addresses the effects of anthropogenic disturbances on genetic diversity and structure of *S. senegal* within Lake Baringo woodland ecosystem, Chapter five addresses effects of anthropogenic disturbances on population structure and natural regeneration patterns of *S. senegal*, Chapter six addresses phenological patterns of *S. senegal* within Lake Baringo woodland, Chapter seven addresses the impacts of anthropogenic disturbances on mating systems in *S. senegal* and Chapter eight addresses the effects of anthropogenic disturbances of gene flow patterns of *S. senegal*. Finally, Chapter nine provides the general discussions of the entire study together with conclusions and major recommendations.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Taxonomy of *Senegalia senegal* (L.) Britton (Syn. *Acacia Senegal* (L.) Willd.)

Senegalia senegal is a leguminous shrub or small tree that sometimes grows to medium size and belongs to the Mimosoideae subfamily of the Fabaceae family (Raddad *et al*., 2005). The tree is member of the genus *Senegalia,* which accommodates about 61 African *Acacia* species (sensu lato) lacking spinescent stipules (Arce & Banks, 2006; Siegler *et al*., 2006). Indeed, the former *Acacia* genera has been assigned to five new genera, *Acacia* sensu stricto (formerly *Acacia* subgenus *Phyllodineae*), *Vachellia* Wight & Arn. (formerly *Acacia* sub-genus *Acacia), Senegalia* (which used to be known as *Acacia* subgenus *Aculeiferum* section *Aculeiferum*), *Acaciella* (which used to be called *Acacia Aculeiferum* section *Filicinae*) and *Mariosousa* (Seigler *et al*., 2006) (species belonging to the *Acacia coulteri* group) (Kyalingalilwa *et al*., 2013). The species grows between 2 m to 15 m in height with relatively flat, round and spreading crown (ICRAF, 1992). The species has several slender and irregular branches with upright twigs (Fagg & Allison, 2004). The bark calor varies between the young and old trees. The trees have yellow or brown back in young trees but later turn dark and generally cracked in the adult stage (ICRAF, 1992). The branches posess prickles at the nodes in groups of three. The middle prickle is characteristically curved downwards while the laterals prickles are hooked upwards (Brenan, 1983). The species have small leaves that are grey-green with alternate and bipinnate arragement. *Senegalia senegal* have white or cream and bisexual flowers that generally occurs on about 7 cm long inflourescence with many stamens (ICRAF, 1992). Pods are brown and normally opens when mature currying as many as 7 seeds (Brenan, 1983; ICRAF, 1992). The seeds are nearly round to flats olive-brown, impressed and horseshoe-shaped (Brenan, 1983).

Senegalia senegal has varietal differences that has been described based on natural distribution, gum arabic chemical composition, molecular and morphological characteristics (Fagg & Allison, 2004). Four distinct varieties are recognized, namely *senegal*, *kerensis*, *rostrata* and *leiorhachis* (Wickens *et al*., 1995). According to Brenan (1983), variety *senegal* shows a wide range of variation in terms of indumentums, armature, flower size and general habit. Variety *leiorhachis* differs from *senegal* solely by its glabrous inflorescence axis, which is actually considered as a minor variation. Variety *kerensis* also seems not to be uniform but its bushy habit is the major distinction in the field. However, the status of these variants of *S. senegal* is still uncertain (Beentje, 1994; Fagg & Allison, 2004). Varieties *kerensis*, *senegal* and *leiorhachis* are found in Kenya (Figure 2.1) but only varieties *senegal* and *kerensis* are present within Lake Baringo woodland ecosystem (Brenan, 1983; Beentje, 1994; Maundu & Tengnas, 2005).

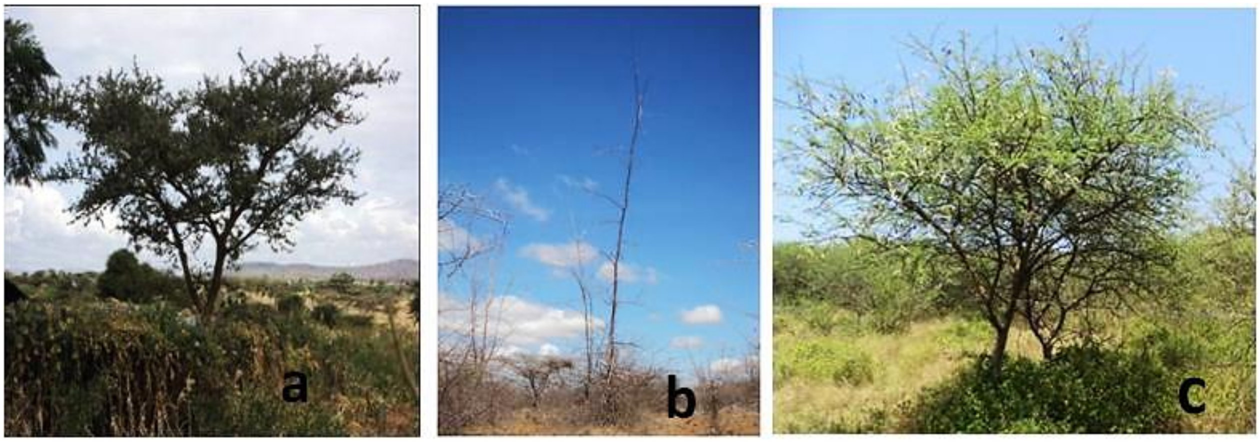

Figure 2.1: *Senegalia senegal* varieties in Kenya; a-variety senegal; b-variety leiorhachis; c-variety kerensis

2.2 Ecological distribution of *S. senegal* in arid and semi-arid lands (ASALs)

Dry land ecosystems cover more than half of the tropics (Muturi, 2012). Generally, these ecosystems are harsh and mainly characterized by high temperatures and deficient in precipitation

(Dangasuk, 1999). They are categorized based on precipitation and evapotranspiration indices into; hyper arid (I), arid (II), semi-arid (III) and sub-humid (IV) (Pauw *et al*., 2000). According to this classification, Lake Baringo woodland ecosystem falls within the ASALs, which consists of class II and III. These fragile ecosystems are degraded and cover about 1.50 x 10^9 hectares of tropics but possess high potential for restoration (Fagg & Allison, 2004). Large percentage of Africa's land surface is ASAL receiving little and erratic annual precipitation ranging between 150-700 mm in seasons as short as 2 to 4 months (Wickens *et al*., 1995). The vegetation types commonly found in these climates are dry forest, *Acacia* bush and woodlands and grasslands and habor tree species such as *S. senegal* (Tandon *et al*., 2001). Annual crop productivity in these areas, especially those with < 400 mm rainfall, is poor and unpredictable (Muturi, 2012). The economic mainstay of people in these ecosystems is livestock production with some dependency on non-timber forest products such as gum arabic sourced from *S. senegal* (Chikamai & Odera, 2002). This has made *S. senegal* to be one of the highly valued species in these ecosystems. In Kenya, ASALs occurs where plant growth is feasible, between 100 and 800 mm of seasonal rainfall and this cover around 87 % of the total land area, which supports about 30 % of the human population and more than 70 % of the livestock population (Muturi, 2012).

Senegalia senegal is drought tolerant and grows in dry areas with annual precipitation falling in the range of 100 - 950 mm, but largely distributed in areas receiving rainfall between 300 - 400 mm, and can survive more than 5 months of dry season of temperatures up to 45 $^{\circ}$C or more (White, 1983; Brenan, 1983; FAO, 1985; Booth & Wickens, 1988; ICRAF, 1992). The species grows well in fossil dunes, loam-sandy soil and Lithosols (FAO, 1985). While the species usually prefers rich and healthy soils, *S. senegal* also grows in poor clay soils such as those reported in South Kordofan and East Sudan (White, 1983). The best growing sites for the species are soils with slight acidity to moderate alkaline pH (Booth & Wickens, 1988). In Africa, the species distribution ranges from north through the central and eastern to the southern parts of the continent (Fagg & Allison, 2004). In Kenya, the species distribution ranges from the very dry northern part of the country down to the coastal region, in habitats that include rocky outcrops, luggas or seasonally dry water courses to well drained fertile loam soil (Brenan, 1983).

2.3 Socio-economic importance of *S. senegal*

Senegalia senegal produces gum arabic, an internationally traded commodity, during the dry season (Wekesa *et al*., 2010). Gum arabic is harvested as a secration from the stems and branches of the tree. These are formed as a result of a process known as gummosis, which is a response to injuries for sealing wounds and protection against extreme heat and desiccation (Fagg & Allison, 2004). The gum is processed and used in food and pharmerceutical industries and other applications such as seed inoculantion in agriculture (Anderson, 1993). Within Lake Baringo ecosystem, no gum arabic exploitation has been reported despite greater potential for commercial production (Lelon, 2008).

The durable, ant resistant dark heavy heart wood of *S. senegal* has found use in local construction of cartwheels, sugar cane crushers, Persian wheel, posts, weaver’s shuttles and agricultural implements (Troup, 1983). In many areas, the wood has been reported to be used in roofing, lining wells, timber frames for huts, and tool handles (Wickens, 1980). In the gum belt, wood is regarded as a secondary product after gum arabic and is usually harvested at the end of gum production cycle after 15-25 years (Fagg & Allison, 2004). The species also offer an important source of fuelwood (Robinson, 1988). The species fuelwood is preferred due to its high calorific value and less smoke. It is estimated that a head-load of *S. senegal* fuelwood, weighing about 22.5 kg, is equivalent to about 72000 Kcal (Baumer, 1990). A study in Niger showed that *S. senegal* fuelwood dry matter has a calorific value of about 20 MJ/kg which is comparable to other fuelwood species such as *Acacia nilotica* (23.4 MJ/kg), *Tectona grandis* (21.7 MJ/kg) and *Cassia fistula* (20.6 MJ/kg) (Fagg & Allison, 2004; Nirmal *et al*., 2009).

In dry seasons, *S. senegal* is valued for fodder production for both livestock and wild herbivores within Lake Baringo woodland (Lelon, 2008). The leaves and pods are very palatable to giraffe, impala, camels, goats and sheep (Booth & Wickens, 1988). The leaves contain digestible proteins (approximately 10 - 13 %) and phosphorus (about 0.12 - 0.15 %). The pods are a source of about 15 % of digestible protein and also contain between 0.12 - 0.14 % phosphorus (IBPGR, 1984). When planted as a hedge for fodder at a spacing of 2.5 x 2.5 m, *S. senegal* is estimated to produce about 3 t ha^{-1} of fresh biomass after 30 months (Booth & Wickens, 1988). For environmental conservation, *S. senegal* plays a significant role in controlling desertification by stabilizing sand dune and acting as windbreaks (FAO, 1985). The species is also known to be very important in ecosystem restoration. In the study by Gebrekirstos *et al*. (2006), *S. senegal* showed better growth performance than the other species in reclamation of degraded lands. This response was attributed to its high predawn water potential (relative easy access to water). The species also possesses extensive lateral and deep taproot patterns making it able to utilize both surface and deep water sources (Abdalla, 2005).

Senegalia senegal agroforestry systems is reported to yield more than when growing trees and agricultural crops seperately in the dry lands. This is mainly due to improved water use through numerous fibrous roots (Abdalla, 2005). Furthermore, *S. senegal* being leguminous also contribute to soil fertility through fixing of atmospheric nitrogen and decomposition of litter fall (Githae *et* *al*., 2011). This species is grown in agroforestry systems specially to produce gum arabic but also for enhancement of the quality of soil (Anderson, 1988). The trees can also be managed to provide fodder. With farming based on *S. senegal* agroforestry, North Kordofan State has been reported to realize considerable agricultural production despite land vulnerability and adverse climatic conditions. Based on the above reports, *S. senegal* agroforestry systems is feasible when considering the multiple productivity of gum gardens in traditional land-use and both national and local economy, and the multipurpose nature of the tree (Abdalla, 2005).

2.4 Breeding systems in *S. senegal*

Breeding system is the way in which a group of organisms are structured in relation to their sexual behavior. The breeding in plants entails the pollen movement from one tree or flower to another and the production of viable seeds for establishment of the new generation. The success of reproduction in plants depends on the availability of flowers, pollen production, reliable pollinators and the viability of the pollen. *Senegalia senegal* tree develop flowers at a young age (about 3 years), producing flowers with white creamy anthers (Stone *et al*., 1998). The flowers are hermaphrodite and in the form of inflorescence with an infra-staminal nectar opening in 1-2 days to release pollen together with nectar (Diallo *et al*., 1997). Flower production in *S. Senegal* differs with the varieties, from before rains to after rains, mostly during the leaf flashing or just before the end of foregoing season growth. The flowers have the style expanded on top of the stamen when the flowers open (Fagg & Allison, 2004). As reported by Stone *et al*. (1998), *S. senegal* flowers are mainly pollinated by bees and wasps but are also visited by other insects such as butterfly. The main insect groups that have been reported to visit *S. senegal* are in the families Coleoptera, Hymenoptera and Lepidoptera (Tybirk, 1993). The flowers open in mid to late morning and anther dehiscence follows immediately. In this regard, the pattern of flower opening is correlated with decreasing relative humidity. In many cases, the trees attract pollinators by availing nectar at the onset of flower dehiscence (Stone *et al*., 1998).

Breeding systems in plants has an important in influencing the amount of genetic variation and how they are distributed within and among populations (Hamrick & Godt, 1989). Several factors have been reported to affect these systems. These include plant population size and density, mode of pollination, availability of pollinators and their foraging behavior (Franceschinelli & Bawa, 2000), flowering synchrony and phenological patterns (Hall *et al*., 1996), the degree of genetic structuring within the populations (Franceschinelli & Bawa, 2000) and the presence of mechanisms that control self-fertilization (Lee *et al*., 2000). Habitat disturbance can also interfere with how the remnant populations are breeding by enhancing reproductive isolation through reduced effective population size or altering the dispersal patterns of pollen (Young *et al*., 1996). The effects of disturbances (reproductive isolation, loss of genetic variability caused by drift and bi-parental inbreeding) would escalate inbreeding frequency in plants (Cruzan, 1998).

Plant species have shown large variation in mating patterns that ranges from highly selfing to exclusively outcrossing. *Senegalia senegal* is reported to be almost exclusively out-crossed and has been shown to be self-incompatible (Tandon *et al.*, 2001). The species produces numerous flowers, which yield abundant pollen grouped into polyads of 16 pollen grains, however, the stigma only accommodates one polyad. Even though self-pollen grain can be deposited on the stigma, the self-incompatibility does not allow any fertilization (Obunga, 1995). As observed in *S. senegal,* most tropical species have been reported to be predominantly outcrossed with outcrossing rates higher than 0.80 (Nason & Hamrick, 1997). A study carried out in Senegal by Diallo *et al*. (1997) found differences in fruit setting in controlled pollination experiment. Percentage of flowers setting fruit varied based on the flower location, for example, 0.12, 0.69 and 1.44 % for flowers within the same inflorescence, same tree and flowers between different trees, respectively. The fruit set from controlled pollination between different trees was comparable to 1.75 % observed for natural pollination. During that study, selfed flowers resulted in lower fruit set than out crossing, demonstrating the importance of out-crossing breeding system to the species. However, inbreeding has been reported to occur in populations of some tropical tree species, which may rise from selfing or mating among related individuals, also known as biparental inbreeding. The latter may reflect the spatial genetic substructure of the population caused by limited dispersal of pollen and/or seeds and/or variation in the flowering synchrony among individuals (Hall *et al*., 1996).

The average seeds per pod in *S. senegal* are three while the mean number of ovules per ovary is normally 11 while mean ratio of seed to ovule is 0.28 (Obunga, 1995). Flower abortions due to competition in the inflorescence and seed predations are the major factors that are reported to limit the survival of seed in the species (Tybirk, 1997). The species produces a papery pod that split open when dry to allow seed dispersal. The seeds are attached to the pod through a thin funicle (Stone *et al*., 1998). Some seeds usually detach themselves from the pods when the funicle dries while some seeds remain attached to the pod and are dispersed with it. Wind is the major seed dispersal agent, although ungulates are also known to disperse seeds (Tybirk, 1993).

2.5 Natural regeneration

Sustainable management of forests and their long term continuity will rely largely on natural regeneration. For example, well managed un-even aged forest will be presented with clearly defined different age classes showing differences is age, height and even diameter. The classes are often distinguished broadly as seedlings, saplings and adult trees. The seedlings are generally defined as the first year regenerates with height less than 10 cm based on field measurements. The plants of height greater than 10 cm are classified as saplings while those with height greater than 10 cm and dbh greater than 5 cm are regarded as adults (Closset-Kopp *et al.*, 2007).

2.5.1 Plant regeneration dynamics

Forest natural conditions are generally affected by chronic anthropogenic disturbances, harsh edaphic conditions and other factors such as topography, light and climate (Kato & Yamamoto, 2002). These factors significantly affect the fluctuation of population size of both seedlings and saplings (Hall & Harcombe, 2001). The regenerates naturally respond to the effect of light by favoring height growth (Kato & Yamamoto, 2002). It is postulated that saplings will normally maintain small sizes and allows shoot growth until favorable environmental condition is available to permit lateral growth (Closset-Kopp *et al.*, 2007). As a response to unfavorable habitat conditions, both seedlings and saplings usually undergoes both morphological and physiological adjustments that would allow them to survive in these environments. However, mortality of plants in the natural ecosystems remains a challenge and one of the most important but least understood aspect of ecological development and production determination (Hamilton, 1986). In their study, Eltahir & Wagner (2012) concluded that environmental factors affect plant population size and structure differently mainly depending on their demographic/life history. In addition to the environmental factors, the availability of sufficient regenerate will largely depend on other factors such as availability of large volumes of viable seeds, favorable climatic and soil conditions (Lamprecht, 1989).

2.6 Plant demographic studies

Demographic studies in plant populations provide useful information on population dynamics and can also be used to examine the biotic and abiotic factors affecting the plant population dynamics for conservation and sustainable use (Tomimatsu & Ohara, 2010). This approach can also be used to study the regeneration of plant species and factors affecting the spatial and temporal patterns in population size structure. The information obtained through demographic studies could thus be useful in restoration of degraded lands. Many researchers emphasise the need for studying and understanding the population dynamics of rare, endangered or key stone species to find the best way of managing and preserving them within or out of their natural habitat (Serbert-Cuvillier *et al*., 2007). The contribution of ecological studies through examinination of demographic differences in relation to temporal and spatial environmental variations, successions and nature management has provided important insights into plant population dynamics (O'Connor, 1994). Matrix modeling is one of the reliable method for the analysis of demographic components of plant population dynamics as described by Alvarez-Buylla *et al*. (1996). The model gives the possibility of identifying the life history stages that have greate impact on population growth and may help in the investigation of plant biological processes that most strongly affect the population and helps in the designing of efficient recovery measures (O'Connor, 1994).

2.7 Plant genetic diversity

Genetic diversity simply means the variability of genes within or among species population and is a key factor in maintaining healthy population of an organism (Jones *et al*., 1997). Genetic diversity can occur in different levels including diversity of the species within ecosystem, populations within ecosystem or individuals within a population (Frankham *et al*., 2011). Due to the interactions of the individuals or populations, a species may develop a structure, which is reflected in the variability distributed within and among natural or artificial populations. Conservation genetics involves utilizing these population genetic theories and techniques to minimize risks of extinction in threatened species (Frankham *et al*., 2011). Maintaining genetic diversity in populations is of importance because it provides a substrate for evolution to occur (Dangasuk, 1999). In other words, the adaptation of an organism to an environmental change requires a pool of variable genes (alleles) in a population, such that the population is able to "respond" effectively to selective pressures (Powel *et al*., 1996). *Senegalia senegal* is widely distributed and is found in varied environmental and edaphic conditions (Fagg & Allison, 2004). This distribution reflects inherent genetic diversity that enhances adaptation to these conditions (Omondi *et al*., 2010b). New alleles in populations are generated by mutations and the frequencies of these alleles are fashioned by the processes of natural selection, migration (or gene flow), and genetic drift (Frankham *et al*., 2011).

Khasa *et al*. (2006) reported that tree populations are composed of individual trees that have different genetic constitution (genotypes) and each individual tree displays a certain range of tolerance to changing environmental conditions (temperature, rainfall, length of the dry period etc.). The main evolutionary factors (genetic drift, mutation, gene flow and natural selection) shaping the genetic structures of populations are theoretically in equilibrium for each species in natural stands. Any significant external influence on one of these factors will disrupt the existing equilibrium, and affect the evolutionary trajectory, hence diminishing the species' adaptive potential of these populations (Young *et al*., 1996). However, tree species possess effective strategies that permit the distribution of genetic information, allowing the trees to retain high genetic diversity (Loveless & Hamrick, 1987). Generally, one can conclusively say that genetic diversity in natural plant populations are linked directly to the individual mating patterns, dispersal of pollen and seeds and the effective breeding population size. The diversity can also be affected by other factors such as geographic distribution and plant community where the species occurs naturally. For sustainable management and conservation of forest ecosystems, detailed inventory of genetic diversity and population structure of species that have undergone disturbance is fundamentally necessary (Kageyama, 1987).

2.7.1 Effects of anthropogenic disturbances on plant genetic diversity

Increasing landscape modifications and land use intensity may break up and decrease native vegetation, which endangers the persistence of species (Debinski & Holt, 2000). The dynamics of land use systems generally lead to significant ecosystem consequences such as forest fragmentation and degradation (Brooks *et al*., 2002). These interferences act by sub-dividing landscapes or populations into smaller units with different population dynamics compared to the original ecosystems. The fragments become isolated and deviates from the original biological processes reflecting “islands of biodiversity” that are bordered by open grounds (Debinski & Holt, 2000). Such disturbances can lead to random fluctuations in allele frequencies within and between populations such that populations become more differentiated from each another. Disturbances may also lead to the rise in inbreeding due to few mates and high probability of common decent amongst individuals (Frankham *et al*., 2011). Principally, the loss of biodiversity and abundances of species is often associated with habitat disturbance and fragmentation (Pardow & Lakatos, 2013). However, if individuals are able to cross the matrix between patches, the spatial disconnect amongst fragments can be overcomed by their functional connectivity.

Tropical trees species such as *S. senegal* are believed to be more threatened by population degradation because of their reproductive patterns and demographic characteristics (Lowe *et al*., 2005). Kala (2000), estimated that within a period less than 50 years, more than 25 % plants would go extinct. The predicted loss is ongoing and is associated with changes in the habitat conditions which affect establishment and persistence of certain species. This is because some species may not be able to persist in small fragments or may be negatively affected by being exposed to the boarder effects (Lovejoy, 1983). It is therefore important to study and understand the effects that have come about due to habitat disturbances so as to help in formulating sustainable management strategies for these ecosystems.

For conservation purposes, it also necessary to identify population whose effective sizes have drastically reduced (bottlenecked populations). These populations may not yet be able to cope with the impacts of disturbances and fragmentation and are generally predisposed to extinction (Newman, 1996). The government of Kenya allowed utilization of *Prosopis juliflora* through charcoal production as a means of controling its invasiveness within Lake Baringo ecosystem. Through this permission, many other species within the ecosystem have been illegally harvested leading to reduction in their population size and are being threatened by extinction (Anderson, 2005). This has been exacerbated by the intensive infrastructural developments and human settlements within the ecosystem. Through over-exploitation of *S. senegal*, the population size might have shrunk significantly hence risks of population bottleneck through genetic drift. Recent population bottlenecks can easily be mitigated by restoration strategies of gene flow into the affected populations. However, the bottlenecked population may have lost rare alleles but may still contain important genetic characteristics (Denniston, 1978; Leberg, 1992). Detection of population bottleneck earlier enough is important in reducing or minimizing the negative genetic effects (Newman, 1996). The task of determining population bottlenecks are, however, hampered by lack of accurate knowledge of the historical genetic variation and population size. For efficient assessment of population bottlenecks, the microsatellite mutation models should be considered before any decision is made (Cornuet & Luikart, 1996).

2.8 Genetic markers

Morphological characterization has been traditionally used in detecting genetic diversity of tree species, however, these morphometric markers are not reliable because they are readily influenced by environmental conditions (Githae *et al*., 2011). Furthermore, inventory is usually done using gene-ecological tests for species undergoing breeding programs. These tests, however, are time-consuming. Due to these drawbacks in using morphometric assessments to determine gebetic diversity, molecular maker systems have been developed. Molecular markers allow rapid assessment of the total genetic diversity in species and guide the sampling of populations representing for preservation. These markers can be functional, especially when genes controlling the characters of interest are known. Molecular markers can also be used to infer signatures of past events, therefore, allowing better strategies for genetic resources preservation.

A genetic marker is a DNA sequence that can be used to differentiate individuals or identify species. The markers are divided into biochemical and molecular markers (Strauss *et al.,* 1992). Biochemical markers are those derived from chemical products of gene expression such as proteins sequences or net charges, and composition of secondary chemicals such as terpenoids (Strauss *et al.,* 1992). Terpenoid compounds include monoterpenes and their derivations, sesqui and diterpenes, polymeric carotenoid and chlorophyll pigments, growth regulators, gibberellic and abscisic acids. Suitability of terpenes as genetic markers in forest genetics has been reviewed by Hanover (1992). The number of potential terpene markers in trees is large owing to thousands of derivatives of isoprene molecule. However, the number of statistically independent dimensions they provide is less than the number of chemical species observed, owing to the strong correlations among terpene concentrations. Despite these drawbacks, terpenoids have proved to be valuable tools to assess geographic variation in forest trees in the years 1960 and 1970, before the widespread of numerical methods for production of phylogenetic trees. Most of terpenoids studies have then been restricted to present maps or matrice distances. Hanover (1992) stated that terpenoid substances in forest trees are versatile biochemical systems for use as genetic markers, although, these markers have not been used in *S. senegal*.

Biochemical markers also include allozymes which are enzymatic electrophoresis, in which allozymes in solution move in response to an electric field. Application of this simple and inexpensive method is universal, and the number of analyses per unit of time is high. However, the number of loci is limited and polymorphism is normally 2-5 alleles per locus. Due to this limitation, large fraction of the diversity may fail to be detected, the nature of genetic difference between alleles is unknown, and possibility of non-neutral polymorphism exists. This method has been widely used in population structure in many tree species including *S. senegal* (Fagg and Allison, 2004).

Molecular genetic markers are those derived from direct analysis of DNA and include both non-PCR and PCR-based markers sequences (Strauss *et al*., 1992). Restriction Fragment Length Polymorphisms (RFLP, PCR-based) is based on the amplification and separation of differences in length of a restricted fragment found segregating in natural populations. The technique involves restricting DNA with endonucleases, separating the resulting fragments by gel electrophoresis (Khasa *et al*., 2006). Indeed, RFLPs result from the presence/absence of particular restriction sites in DNA, and they are abundant in the genomes of most organisms. The markers have been applied in several genetic diversity studies of natural populations, as they are highly discriminating and only 5-6 loci need to be tested. However, this inexpensive technique requires high quality and large amount of DNA, is slow and cannot be automated, and statistical analysis is difficult. Also, larger restriction fragments in plants are more likely to be polymorphic than smaller fragments owing to the high frequency of insertions/deletions observed at these loci (Miller & Tanskley, 1990). However, RFLPs analyses have been appliedin determination of genetic structure in plants such as *Thuja plicata* (Raybould *et al*., 1996). Restriction analyses techniques are also used in plant organelle DNA e.g., plant mitochondrial DNA and chloroplast DNA.

Invention of PCR technology has facilitated the development and use of various other genetic markers, including Random Amplified Polymorphic DNA (RAPDs), mitochondrial, plant chloroplast DNA (mtDNA and cpDNA, respectively), Amplified-Fragment Length Polymorphism (AFLPs), Single-Strand Conformation Polymorphisms (SSCPs), minisatellites, simple sequence repeats (SSRs) or microsatellites, Expressed Sequence Tag Polymorphism (ESTP), Expressed Sequence Tags-Simple Sequence Repeats (EST-SSR), candidate genes, single nucleotide polymorphisms (SNPs) and DNA sequencing. These markers can be of nuclear or organelle origin e.g., mtDNA (Avise, 2004). Polymerase Chain Reaction entails the replication of specific DNA sections severally using oligonucleotide primers (small number of nucleotides joined together in a short stretch of single-stranded DNA). RAPD in combination with ISSR (Chiveu *et al*., 2008), ISSR in combination with cpSSR (Githae *et al*., 2011), AFLP (Sirelkhatem & Gaali, 2009) and nSSR (Omondi *et al*., 2010b) markers have been utilized in *S. senegal* population genetic studies reporting high genetic diversity. Among these markers, SSR markers have proved to be reliable due to their reproducibility and high polymorphism, hence was chosen for use in this study.

In a recent review, Lateef (2015) classified molecular markers in three categories: (i) Low-throughput marker systems; (ii) Medium-throughput marker systems and (iii) High-throughput marker systems of next generation sequencing (NGS). Low-throughput marker systems include RFLPs, also known as first generation molecular markers mainly used in 1980s and 1990s in plant genetic studies. Medium-throughput marker systems or second generation molecular markers include RAPDs, AFLPs, minisatellites (also referred as VNTRs), Microsatellites or SSRs, cleaved Amplified Polymorphic sequences (cAPs). High-Throughput Marker Systems or third generation markers include SNPs, KBioscience Competitive Allele-Specific PCR (KASPar) and Genotyping-by-Sequencing (GBS). The use of these markers in tree improvement (gene conservation, assembly of breeding populations, genotype verification and delineation, taxonomy and phylogenetic studies, pollen contamination, mating systems, genetic fingerprinting, marker aided selection) has been reviewed by Burdon & Wilcox (2011) and Mahajan & Gupta (2012).

CHAPTER THREE

3.0 GENERAL MATERIALS AND METHODS

3.1 Description of the study area

The study was carried out within Lake Baringo woodland ecosystem where *S. senegal* is found and possess potential for economic utilization. The Lake Baringo woodland ecosystem is found between latitudes 00°08 ′ South and 00°45´ North and longitudes 035°50´ and 036°15´ East (Figure 3.1).

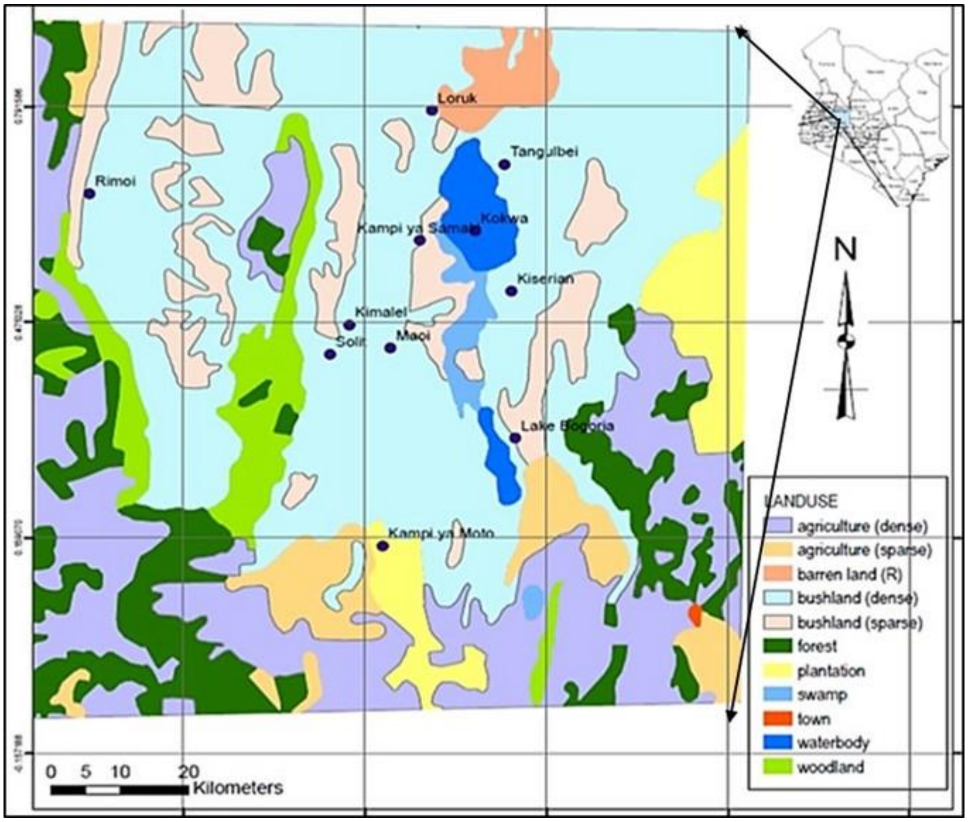

Figure 3.1: Lake Baringo woodland ecosystem showing *S. senegal* study populations and land use patterns.

The ecosystem is quite heterogeneous in terms of topography (characterized by many small hills but majorly step faulted Rift-Valley floor), soils, vegetation and climatic conditions, creating a highly diverse landscape with varying potentials for different land use activities (Sombroek *et al.*, 1982; Mwasi, 2004). The ecosystem lies in the eastern (Gregory) arm of the African Rift Valley. Most of the study area is in the floor of this Rift valley system, bounding Lake Baringo to the North and Lake Bogoria to the South. The eastern and western boundaries are marked by the Laikipia escarpment and the Tugen hills respectively, which rise from altitudes of 1000 m to over 2500 m above sea level (a.s.l) passing through a heavily dissected basalt plateau (Mwasi, 2004). The temperatures are fairly warm to hot with the mean annual temperatures ranging from 22 - 24 °C. The mean maximum and minimum temperature ranges from 28 - 30 °C and 16-18 °C, respectively. The ecosystem falls under semi-arid ecological zone with a bi-modal rainfall pattern, rains occurring mainly in April-June and October-December (Sutherland *et al.*, 1991). Mean annual precipitation ranges from 250 - 900 mm with mean annual potential evapo-transpiration ranging from 1650-2300 mm.

The ecosystem is characterised by thorny bush savannah with *Acacia tortilis, A. seyal, S. senegal* and scattered *Balanites aegyptiaca* trees. Patches of less woody *Acacia reficiens* and *A. nubica* dominate some areas. The trees on these plains are partly fed by ground water and thus not very sensitive to seasonal rainfall. However, the undergrowth vegetation, consisting of mixed grasses and forbs, is extremely sensitive to the availability of soil water and thus changes from a complete cover to virtually bare surface over the seasons. The vegetation above the plains consists of a mixture of woody acacias *(Acacia mellifera, A. nilotica and A. gerardii*) and other plants including *B. aegyptiaca* and *Boscia angustifolia*, which form the key vegetation stratum. The under-storey vegetation consists of annual forbs such as *Acatipha fruticosa* and perennial shrubs such as *Croton dichogamus, Dodoma viscosa* and *Psidia arabica* (Mwasi, 2004).

3.2 Degradation and fragmentation history of the study area

Lake Baringo woodland ecosystem has undergone systematic vegetation degradation through human disturbances over the years (Kiage *et al*., 2007). This has led to deforestation and fragmentation of the ecosystem into patches. The deforestation and fragmentation is estimated to have begun about 350 years ago when the forests and woodlands were being converted into large scale agricultural production (Kiage & Liu, 2009). This included the introduction of large scale sisal plantations and irrigation agriculture that led to clearance of large chunks of the forests and woodlands. Furthermore, vegetation change study by Kiage *et al*. (2007) revealed that within a span of only 14 years, between the years 1986 to 2000, about 40 % of the vegetation within the ecosystem had been lost due to human disturbances. Other studies estimated that Lake Baringo ecosystem actually lost over 50 % of its vegetation between the years 1976 to 2000 (Kiage & Liu, 2009). Since then, more destruction and fragmentation of the remaining vegetation has been going on, therefore, the proportion of forest and woodland lost to date might be much higher than the 50 % already reported. In addition to agricultural cultivation, other disturbances that have occurred within the woodland include livestock overgrazing, charcoal production, industrialization and urban development (BCG, 2014). Furthermore, human population within the ecosystem has grown tremendously since the year 1979 from 220,922 to 302,245 in 2004 with a growth rate of about 2.65 % per annum (Kiage *et al*., 2007). Similar trend of population growth has been observed in livestock by Hickley *et al*. (2004). These increases in both human and livestock population densities have added more pressure to the limited vegetation cover of the ecosystem and have negatively affected important tree species therein including *S. senegal*. The disturbances have created several isolated fraction of the forests and woodland, however, no study has been conducted to estimate their sizes. Through extensive survey of the ecosystem by Lelon (2008), 11 natural populations of *S. senegal* e.g. Maoi, Kimalel, Solit, Kampi ya Samaki, Lake Bogoria, Tangulbei, Loruk, Kiserian, Kokwa, Kampi ya moto and Rimoi have been identified (Figure 3.1).

3.3 Development of population disturbance index (PDI)

To determine the effects of anthropogenic disturbances within the ecosystem, the populations were assessed and categorized in to disturbance levels. Within each of the 11 populations, 15 plots measuring 20 × 20 m were established a long 100 m by 10 km transect at 200 m intervals for the assessments and documentation of disturbance indicators. The indicators of disturbances assessed were; number of new settlements, presence of grazing livestock, browsed seedlings cut tree stumps, charcoal kilns, fenced plots and manmade water pans. The quantities of these indicators were then converted into percentages of the totals to reflect their intensities within the populations. The indicators were then combined into a population disturbance index (PDI) as described by Bynum, (1999). Population disturbance index for each population was calculated as the average of all present indicators. The populations were then classified as heavily disturbed or lightly disturbed. Populations with PDI equals to or greater than 40 % ($PDI \geq 40$) were considered as heavily disturbed while those with PDI less than 40 % ($PDI < 40$) were regarded as lightly disturbed. The choice of indicator variables was informed by the literature concerning anthropogenic activities commonly found affecting vegetation ecology within the ecosystem as reportd by Baringo County Government (BCG) (2014).

3.4 DNA isolation and Polymerase Chain Reaction (PCR) analyses

Modified cetyl trimethyl ammonium bromide (CTAB) method as described by Hanaoka *et al*. (2012) was used in extraction of DNA from silica gel dried leaf materials. About 0.2 g of the dry leaf tissues were ground into fine powder using mixer mill and then transferred to a 2.0 ml microfuge tube containing 1,000 µl isolation buffer (IB, 10 % polyethylene glycol, 0.35 M sorbitol, 0.1 M Tris-HCl-pH 8.0, 0.5 % spermidine, 0.5 % spermine and 0.5 % β-mercaptoethanol). The solution was mixed thoroughly by vortexing then centrifuged at 12,000 rpm and 4 °C for 3 min. The supernatant was removed and another 1,000 µl IB added, vortexed and centrifuged. This step was repeated 3 times till the supernatant was less viscous. The supernatant was removed and 300 µl lysis buffer (0.35 M sorbitol, 0.1 M Tris-HCl-pH 8.0, 0.5 % spermidine, 0.5 % spermine and 0.5 % β-mercaptoethanol) added and mixed by vortexing. 30 µl sarcosine solution was added and the solution incubated at room temperature for 10 min. 300 µl 2x CTAB buffer (2 % CTAB, 0.1 M Tris-HCl, 20 mM EDTA, 1.4 M NaCl, 0.5 % β-mercaptoethanol) was added to the solution and then incubated at 65 °C for 30 min in oscillating water bath. Equal volume (600 µl) of CIA (chloroform isoamyl alcohol; 24:1 ratio) was added and mix by gentle inversion for 20 min and centrifuged (14,000 rpm at room temperature) for 10 min. The supernatant was then transferred to a new 1.5 ml microfuge tube and 600 µl isopropanol added. The solution was mixed by gentle inversion and centrifuged (15,000 rpm at 4 °C) for 5 min.

The supernatant was then discarded and 500 µl TE (10 mM Tris-HCl and 1 mM EDTA) added to dissolve the precipitated nucleic acid. 10ng/µl of RNase (following the protocol of the reagent supplier) was added to the solution and mixed by inversion then incubated at 37 °C for 60 min. Equal volume (500 µl) of TE-saturated phenol was added and the solution mixed by gentle inversion for 10 min then centrifuged (8,000 rpm at room temperature) for 10 min. The supernatant was transferred to a new 1.5 ml microfuge tube. Equal volume (500 µl) of TE-saturated phenol was added and the solution mixed by gentle inversion for 20 min then centrifuged (8,000 rpm at room temperature for 10 min). The supernatant was transferred to a new 1.5 ml microfuge tube and 1/10 volume (50 µl) of 3 M ammonium acetate added then equal volume (550 µl) of isopropanol added and the solution mixed by gentle inversion then centrifuged (15,000 rpm at 4 °C) for 5 min. The supernatant was discarded and 800 µl of 70 % ethanol added and flipped then centrifuged (15,000 rpm at 4 °C) for 5 min to wash the DNA pellet. The supernatant was discarded and the DNA pellet dried for 15 min and dissolved in 200 µl DNase-free water in readiness for use.

The PCR analysis of 12 microstellite loci (Table 3.1) was performed in a final volume of 10 µl containing 2x Multiplex PCR Master Mix (Qiagen), 0.15 µM non-flourescently labeled forward primer (Applied Biosystems), 0.01 µM flourecently labled forward primer and 0.15 µM reverse primer. The non-flourescently lebeled forward primer was used in the reaction for stabilization purposes. Approximately 10 ng of template DNA was finally added. The reactions for all the loci were performed using a touch down procedure. The first cycle started with an initial denaturation at 95°C for 15 min, 67 °C for 1.5 min and 72 °C for 1 min, followed by 8 cycles at 94 °C for 30 s, 65 °C for 1.5 min with 2°C decrease after every cycle and 72 °C for 1 min followed by 24 cycles at 94 °C for 30 s, 51 °C for 1.5 min and 72 °C for 1 min, and a single final extension at 60 °C for 30 min. The reactions were performed using Verity 96 well thermocycler (Applied Biosystems). Amplified fragments were analysed through capillary electrophoresis against an internal standard (Liz 600 size standard) on an ABI 3500 genetic analyser (Applied Biosystems). The alleles were scored using GeneMapper version 5 software (Applied Biosystems).

Table 3.1: Description of 12 *S. senegal* microsatellite markers used in genetic diversity, mating systems and gene flow studies in Lake Baringo woodland ecosystem

Locus name	Accession no.	Motif repeat	Primer sequence (5′-3′)	Allele size range (bp)
mAsCIRB09	FM883654	(TA)3 TG (TA)3 TG (TA)3	F: CCTTATGAACCAAAACCAGC-PET R: CAACGAGCATTAACACCAAC	297-341
mAsCIRB10	FM883648	(TA)7 (N)6 (CA)5 (N)6	F: TTTCTCCCCATAAATCGTGC-NED R: TTATCCGTTTGCTTGCCG	163-197
mAsCIRC07	FM883653	(TG)9 TC (TA)3	F: TAATCGTCCGTAGCAAG-PET R: TCCTATTCTCCTCCCTC	148-172
mAsCIRE06	FM883647	(GA)8 G (GA)7	F: CTGTGATGGATAACCTTTTG-FAM R: TCCTCTTCCTCCTTCTCTC	168-190
mAsCIRE07	FM883644	(GGA)2 (GAA) (GGA)4	F: GAAGCAGAAGCAGAAGCAGC-PET R: CCCCCCTCACACTCATCTC	148-163
mAsCIRE10	FM883645	(GAT)6	F: GAGAAACTGGAGAGGGGAAG-NED R: GCGACAAAAGTAGTAAAGGGC	117-132
mAsCIRF03	FM883651	(TA)4 (N)55 (AC)10	F: CACTTTTACTTTGTGATCTCCC-PET R: CAGTCTTGTGTGCGTCTTAC	272-336
mAsCIRH01	FM883646	(TG)9 TA CA (TA)3	F: CAGAGGTTCAGGTATATGTGTG-NED R: TTGTGTTAGTTTCAGATGGC	192-236
mAsCIRH09	FM883649	(GAA)6	F: CCACTCCAGGTACAAATCAC-FAM R: AGCCAGCAGAAAACTTAGG	135-156
Ab26	AY843557	(AG)8 (AG)9	F: ATATTCTGCTTTAGTCTA-NED R: GGGGCATAAATATGAG	126-149
Ame03	DQ467673	(AG)9	F: GAACAATATCAGCAATCACT-PET R: CCTCATGCACACACAAGAT	137-162
Ame07	DQ467658	(GT)20	F: TAAAAACAAAAACCCAACTAAATG-FAM R: GTCCAAAACTCTTCAATGTCAA	342-388

*The forward primers were fluorescently labeled with PET, NED and VIC

CHAPTER FOUR

4.0 GENETIC DIVERSITY AND POPULATION STRUCTURE OF *Senegalia senegal* WITHIN LAKE BARINGO WOODLAND ECOSYSTEM

4.1 Introduction

Many studies have reported spatial patterns of genetic variation in several tree species with diverse life history associated with landscape features, especially those related to recent human disturbances (Lowe *et al.,* 2005). These studies offer evidence on how landscape and ecological factors influence population genetic (Storfer *et al*., 2007). Such studies are important more so to the dryland tree species that are under human disturbance threat but has significant potential to improving local livelihoods such as *S. senegal*. Possible theoretical impacts generated by different types of human activities would suggest that forest degradation and separation of natural populations may decrease the flow of genetic information and effective species population size (Fahring, 2003). This will significantly affect the species evolutionary potential and fitness to survive the ever changing climate. Generally, if the remaining populations are isolated for many generations, forest fragmentation may lead to continuous allele loss and consequently, there will be elevated genetic diferentaition among more isolated populations (Couvet, 2002; Pither *et al*., 2003). However, contrary to the above argument, some studies have shown that habitat disturbance and fragmentation sometimes facilitate both pollen flow and long-distance gene dispersal (Dick *et al.,* 2003; Bacles *et al.,* 2006). Thus, the effects of population disturbance on the genetic characteristics of tree are varied and multifaceted than may have been believed (Lowe *et al.,* 2005).

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Studies at landscape level scales provide valuable information on evolutionary patterns by revealing the movement patterns of genetic information at different scales (Storfer *et al*., 2007). In heavily disturbed forests, population genetic studies indicate species bottlenecks, through reproductive dominance, reduced genetic diversity of a founder population and increased levels of inbreeding (Sezen *et al.,* 2005). To recover, such forests will require continuous gene flow over successive generations to restore genetic diversity to levels currently observed in older and more established forests (Sezen *et al.,* 2005). Several studies have evaluated the landscape context in disturbed tropical rain forests, however, few works have been focused in the dry forests ecosystems (Ally & Ritland, 2007).

The aim of this study was to determine the patterns of genetic diversity and population structuring among fragments (remnant populations) of *S. senegal* within Lake Baringo woodland ecosystem based on anthropogenic levels.

4.2 Materials and methods

4 .2.1 Samples collection

All the 11natural populations found within Lake Baringo woodland ecosystem were involved in this study. Leaves were collected from 330 individual trees of *S. senegal* distributed within the 11 populations. From each population, 30 individual trees were selected randomly for genotyping. The trees were separated by about 100 m distance apart to evade picking individuals that are related genetically. From each tree, young and healthy leaf tissues were harvested and preserved in silica gel until the time for DNA isolation. The package was marked with the sample number and the source population. The GPS positions of the sampled trees were also recorded.

4.2.2 DNA analysis

Isolation of DNA was undertaken as described in Chapter 3. The extracted DNA was analysed and genotyped using 12 microsatellite markers (Table 3.1) as described in Chapter 3. Genotypic frequencies for conformance to Hardy-Weinberg Equilibrium (HWE) were assessed followed by test of linkage disequilibrium between makers prior to the estimation of genetic diversity parameters using Fisher's exact test available in GenePop version 3.4 (Raymond & Rousset, 1995). Significant levels were determined using the Markov chain method. Sequential Bonferroni correction was used to determine significance level for all the tests with an initial *P*-level of 0.05 (Holm, 1978). Polymorphic information content (PIC) which is used as a marker discriminatory power, was was calculated as;

$PIC = 1 - \sum P^2 i$ where P_i is the frequency of the *i*th allele at a given locus.

Genetic diversity was determined per population and overall as the average number of alleles per locus (*A*), Number of alleles per population (N_A), number of effective alleles per population (A_E), allelic richness per population (A_R), observed proportion of heterozygotes (H_O), expected proportion of heterozygotes or gene diversity (H_E), and *F*- statistics (F_{IS} and F_{ST},) were determined using FSTAT version 3.9.3.2 (Goudet, 2002). The probability of the *F*-statistics being greater than zero was determined by bootstrap analysis using 1000 replicates, with a 95 % confidence interval. The indirect outcrossing rate was calculated as; $(1-F_{IS})/(1+F_{IS})$ (Weir, 1996).

Multilocus estimate of the number of migrants per generation (*Nm*) between populations was determined trough the private allele method described by Slatkin (1985). The differences among populations and levels of disturbance for diversity parameters (number of alleles, heterozygosity, allelic richness) and fixation index were studied by one-way analysis of variance and followed by paired t-test for mean comparison (Tapio *et al*., 2003). Bonferroni corrections were applied during the tests. These analyses were performed using the XLSTAT software version 2008.4.01 (Addinsoft, 2008).

The extent and significance of genetic differentiation among populations was quantified using unbiased estimates of R_{ST} based on stepwise mutation model (Kimura & Ohta, 1978; Slatkin, 1995). Unbiased estimates of R_{ST} and their significance were determined after 1000 bootstraps with 95 % nominal confidence interval and permutation tests of 10,000 permutations (Lynch & Crease, 1990), using R_{ST}CALC version 2.2 (Goodman, 1997). Analysis of molecular variance (AMOVA) was calculated through Arlequin software version 1.1 (Schneider *et al*., 2000), in which significance level for the overall values were determined after 1023 permutation.

Population structure was analysed using Bayesian inference in STRUCTURE software version 2.2, (Falush *et al*., 2007). This method assigns individuals to populations and identifies migrants and admixed individuals. The number of gene pools (structures), *K*, was set from 1 to 11. After preliminary testing, the optimal number of groups was determined using 50,000 cycle burn-in period and 500,000 Monte-Carlo Markov Chains without earlier knowledge of the population where each individual samples came from, based on the admixture model (which assumes that individual sampless may be having mixed decent), assuming correlated allele frequencies among subpopulations and without any prior information on clustering of samples. Simulations for each value of K were repeated 20 times to provide stable probability estimates. The optimal number of groups was determined using the second order rate of change approach as described by Evanno *et al*. (2007). Cluster analysis was performed using UPGMA algorithms and dendrogram constructed using MEGA6 software based on 1000 bootstrap samples (Kumar *et al*., 2004). Principal coordinate analysis (PCoA) in GenAlex software version 6 (Peakall & Smouse, 2006) was also used to determine the pattern of genetic relationship of the populations.

Spatial genetic structure was also determined by analysing the samples for isolation by distance. Pairwise geographic distance matrix was calculated using the geographic positions (latitude and longitude) of each population, using the Geographic Distance Matrix Generator version 1.2.2 (Ersts http://biodiversityinformatics.amnh.org/open_source/gdmg). The hypothesis that populations are differentiated because of isolation by distance (Wright, 1943) was calculated by correlating R_{ST} matrix against the geographical distance matrix. Spearman's rank correlation coefficient was calculated and significance determined with 10,000 permutations at $P = 0.05$ using a Mantel procedure (Mantel, 1967) presented in GenePop software version 3.4 (Raymond & Rousset, 1995).

The populations were assessed for possible recent bottleneck events, following the procedure described by Cornuet & Luikart (1996), using BOTTLENECK program version 1.2 (Piry *et al.*, 1999). The infinite alleles model (IAM), stepwise mutation model (SMM) and two-phase mutation model (TPM, using 95 % SMM) were applied. The models compare observed and expected gene diversities using the observed number of alleles under mutation-drift equilibrium to test for compatibility with an equilibrium model (Cornuet & Luikart, 1996). Tests for mode-shift (change in allele frequency distribution) were also applied. If a recent bottleneck has occurred, allelic diversity is likely to have been lost faster than heterozygosity and may be manifested as a shift in the allele frequency distribution away from rare/low-frequency alleles (Piry *et al.*, 1999).

4.3 Results

4.3.1 Population disturbance index (PDI)

The PDI for all the populations are as shown in Table 4.1. Out of the 11 populations, five were classified as lightly disturbed (PDI < 40) while six populations were classified as heavily disturbed (PDI ≥ 40).

Table 4.1: Anthropogenic indicators and population distabance ranks within Lake Baringo woodland ecosystem

	Anthropogenic indicator							
Population	CTS	PCK	PGL	PSB	PNS	NFL	PWP	PDI (%)
Tangulbei	16.2	00.0	00.0	48.3	00.0	00.0	00.0	09.2a
Kimalel	48.1	33.3	20.6	77.1	32.3	41.6	42.4	42.2b
Kampi ya Samaki	53.6	22.9	24.6	85.7	47.8	38.8	58.1	47.4b
Lake Bogoria	12.8	00.0	00.6	15.6	00.0	00.0	00.0	04.1a
Maoi	33.1	8.6	19.4	62.2	52.3	44.1	62.5	40.3b
Solit	42.3	14.3	11.8	72.4	41.9	35.9	76.2	42.1b
Kokwa	22.7	00.0	01.0	28.1	00.0	00.0	00.0	07.4a
Kiserian	18.3	05.7	00.0	00.0	00.0	00.0	10.1	04.9a
Loruk	12.1	00.0	00.0	00.0	00.0	00.0	16.6	04.1a
Rimo	38.6	11.4	21.2	81.7	38.3	45.3	78.2	45.0b
Kampi ya Moto	62.5	08.6	36.5	86.2	56.1	56.8	64.6	53.0b

Abbreviations: CTS, Cut tree stumps; PCK, Presence of charcoal kilns; PGL, Presence of grazing livestock; PSB, Percentage seedlings browsed; PNS, Presence of new settlements; NFL, New fenced land; PWP, Presence of water pans; PDI, population disturbance index (%) a, lightly disturbed; b, heavily disturbed.

4.3.2 Population genetic diversity measures

In a total of 330 individual of *S. senegal* surveyed, 164 alleles were identified at 12 microsatellite loci. All the studied microsatellite loci were variable and informative with the polymorphic information content (PIC) ranging from 0.44 (mAsCIRB09) to 0.92 (mAsCIRF03). The mean number of alleles per locus was 13.7 and ranged from 5 (mAsCIRE07 and mAsCIRB09) to 27 (mAsCIRF03) (Table 4.2).

Table 4.2: Descriptive statistics of 12 microsatellite loci studied over all the 11 populations of *S. senegal* within Lake Baringo woodland ecosystem

*Locus**	*A*	H_O	H_E	F_{IS}	F_{ST}	R_{ST}	*Nm*	*PIC*
mAsCIRH09[a]	16	0.834	0.804	-0.037	0.047	0.007	5.066	0.84
mAsCIRE10[a]	7	0.642	0.601	-0.069	0.066	0.078	3.555	0.76
mAsCIRE07[a]	5	0.043	0.045	0.063	0.050	0.064	4.735	0.45
mAsCIRH01[a]	23	0.744	0.809	0.080*	0.052	0.076	4.530	0.87
mAsCIRF03[a]	27	0.749	0.839	0.107*	0.087	0.517	2.608	0.92
mAsCIRB09[a]	5	0.042	0.040	-0.043	0.026	0.000	9.290	0.44
mAsCIRE06[a]	18	0.644	0.753	0.144*	0.123	0.616	1.789	0.86
mAsCIRB10[a]	15	0.988	0.733	-0.047	0.078	0.148	2.956	0.80
mAsCIRC07[a]	20	0.775	0.819	0.053*	0.078	0.197	2.972	0.89
Ab26[b]	9	0.763	0.779	0.054	0.041	0.153	2.110	0.78
Ame03[b]	11	0.738	0.811	-0.075	0.081	0.197	3.020	0.86
Ame07[b]	8	0.688	0.624	0.007	0.069	0.173	3.140	0.83
Mean	13.7	0.638	0.639	-0.005	0.067	0.186	3.814	0.78

Significance levels of F_{IS} departure from zero is $*P < 0.001$; [a]Assoumane *et al*. (2009); [b]Omondi *et al*. (2010a); *A*, total number of alleles per locus; H_E, expected proportion of heterozygotes; H_O, observed proportion of heterozygotes; F_{IS}, the inbreeding coefficient; F_{ST} and R_{ST} population differentiations; *Nm*, number of migrants per generation; *PIC*, polymorphic information content

The diversity parameters varied across the populations as shown in Table 4.3. The average number of alleles observed per locus per population was 7.42 ranging from 6.65 (Tangulbei) to 8.32 (Kampi ya Samaki) (Table 4.3). For the microsatellite loci used, the mean observed proportion of heterozygosity (H_O) was 0.638 and ranged from 0.042 (mAsCIRB09) to 0.988 (mAsCIRB10), while mean expected proportion of heterozygosity (H_E) was 0.639 ranging from 0.040 (mAsCIRB09) to 0.839 (mAsCIRF03) (Table 4.2). Among the 11 populations, the highest H_E (0.644) was recorded in Kokwa population with the lowest value of $H_E = 0.582$ observed in Rimoi population. The highest value of H_O (0.707) was recorded in Kampi ya Moto population with the lowest value of 0.569 in Lake Bogoria (Table 4.3).

4.3.3 Linkage disequilibrium and Hardy-Weinberg equilibrium

No significant linkage disequilibrium was detected between different genotypes at each of the microsatellite loci except between mAsCIRE10 and mAsCIRB10. Hardy-Weinberg equilibrium (HWE) was measured using *F*-statistics and their departure from zero tested. Out of the 11 populations, only one population (Kampi ya Moto) had significant departure from HWE, corresponding to excess heterozygotes (Table 4.3).

Based on loci, the estimates of inbreeding coefficient (F_{IS}) deviation was due to a deficit of heterozygotes (Table 4.2). However, based on the populations, the estimates of F_{IS} deviation was due to heterozygosity excess (Table 4.3). No linkage disequilibrium was observed among the loci after undertaking Bonferroni correction, indicating that all the 12 loci segregate independently of each other and was appropriate for structure analysis. The indirect estimates of average outcrossing rates within the ecosystem ranged from 0.794 (Kiserian) to 0.999 (Kampi ya Moto) with a mean of 0.997 indicating a prredomonant outcrossing mating pattern (Table 4.3).

Table 4.3: Descriptive statistics over all loci for 11 natural populations of *S. senegal* within Lake Baringo woodland ecosystem assessed in September 2016

Population	Latitude	Longitude	Altitude	N	N_A	A_E	A_R	H_O	H_E	F_{IS}	$\%P$	t
Kiserian	0.52069	36.1215	1020	30	7.431	4.183	7.01	0.600	0.637	0.116	89.01	0.794
Kokwa	0.60928	36.0743	1029	30	7.674	4.261	7.21	0.631	0.644	0.001	100.00	0.999
Kampi ya Moto	0.14697	35.9534	1806	30	6.659	3.664	6.3	0.707	0.584*	-0.223*	77.90	0.999
Loruk	0.78564	36.0181	0966	30	8.209	4.569	7.91	0.617	0.64	0.037	89.01	0.931
Kampi ya Samaki	0.59556	36.0020	1019	30	8.320	4.996	7.99	0.578	0.624	0.075	77.90	0.863
Maoi	0.43744	35.9633	1130	30	7.765	4.646	7.44	0.576	0.617	0.038	100.00	0.93
Lake Bogoria	0.29742	36.0812	1003	30	6.658	3.963	6.29	0.569	0.599	0.039	77.90	0.928
Tangulbei	0.69417	36.0972	1108	30	6.654	3.928	6.46	0.644	0.638	-0.031	100.00	0.999
Rimoi	0.66344	35.5704	1154	30	6.987	3.505	6.55	0.588	0.582	-0.005	89.01	0.999
Solit	0.42809	35.8846	1291	30	7.431	3.706	6.98	0.632	0.606	-0.051	77.90	0.999
Kimalel	0.47081	35.9100	1259	30	7.876	3.718	7.38	0.655	0.613	-0.078	100.00	0.999
Average				30	7.421	4.104	7.05	0.618	0.617	-0.006	89.01	0.997

Level of significance of departure from zero is $*P < 0.001$; N, number of samples; N_A, average number of alleles; A_E, effective number of alleles; A_R, allelic richness; H_E, expected proportion of heterozygotes; H_O, observed proportion of heterozygotes; F_{IS}, the inbreeding coefficient; $\%P$, percentage polymorphism and t, outcrossing rate. HWE exact test was done using Markov chain after 100,000 interactions with a forecasted chain length of 100,000

The results of the weighted ANOVA test for the human disturbance level effect, based on diversity indices showed a significant difference among the two levels of disturbance for the mean number of allele (N_A), allelic richness (A_R) and expected heterozygosity (H_E). The N_A ($F_{1,\,10} = 10.57$; $P < 0.05$), H_E ($F_{1,\,10} = 6.30$; $P < 0.05$) and A_R ($F_{1,\,10} = 9.30$; $P < 0.05$) were higher in populations with light disturbance at the 5% level than heavily disturbed populations (Table 4.4).

Table 4.4: One-way analysis of variance of the effects of human disturbance on genetic diversity of *S. senegal* within Lake Baringo woodland ecosystem

Level of disturbance	N_{pop} (N_{ind})	N_A	A_R	H_E	F_{IS}
Heavy	6 (180)	7.02a	6.56a	0.577a	-0.037a
Light	5 (150)	7.31b	6.72b	0.635b	0.026a
DF	-	1,10	1,10	1,10	1,10
F	-	10.57	9.30	6.30	1.18
P	-	0.002	0.016	0.001	0.175

*****values followed by the same letter shows no significant difference at 5% level after bonferroni correction; N_{pop}, number of populations; N_{ind}, number of individuals; *NA*, number of alleles; *AR*, allelic richness; H_E, gene diversity; F_{IS}, fixation index

4.3.4 Population differentiation and genetic structure

The overall F_{IS} value was 0.006 ($P > 0.05$), indicating insignificant inbreeding among the individuals. In order to measure the degree of population differentiation, two model theories were tested; the infinite alleles model estimate (F_{ST}) and the stepwise mutation model estimate (R_{ST}). The overall values of genetic differentiation for each model produced different but significantly different ($P < 0.001$); $F_{ST} = 0.059$, $R_{ST} = 0.175$. These populations, however, had overall large estimates of migrants ($Nm = 0.997$) indicating presence of some flow of genetic information among the populations. The AMOVA also showed significant overall genetic divergence among the populations. Of the total molecular variance, 11.4 % (V = 0.772; $P < 0.001$) was due to differences among populations. Differentiation within populations was high (88.6 %) and significant (V = 6.011; $P < 0.001$).

The result of STRUCTURE analysis is shown in Figure 4.1 using the delta K (ΔK) criterion. Bayesian clustering suggested the presence of three groups (gene pools) as most probable, although most individuals showed mixed ancestry. With $K = 3$, the same population structure was consistently obtained for the 20 independent runs. This separation placed Kampi ya Moto, Rimoi and Solit populations within one group (defining group membership as most individuals within a population having > 60 % ancestry of that group), Kiserian, Kokwa and Kimalel in group two while Loruk, Kampi ya Samaki, Maoi, Lake Bogoria, and Tangulbei in the third group (Figure 4.1). The genetic groups were, however, not spatially separated.

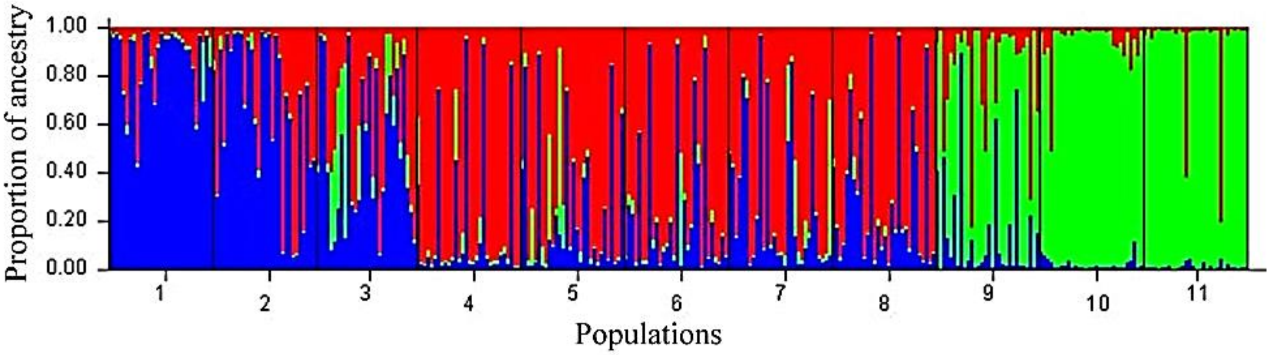

Figure 4.1: Plots of proportional group membership for 330 *S. senegal* tree genotypes within Lake Baringo woodland ecosystem at 12 nuclear microsatellite loci, for $K = 3$. Each line represents a single tree, with colour representing proportion of ancestry derived from each group. Gray lines indicate the division. Populations are 1, Kiserian; 2, Kokwa; 3, Kampi ya Moto; 4, Loruk; 5, Kampi ya Samaki; 6, Maoi; 7, Lake Bogoria; 8, Tangulbei; 9, Rimoi; 10, Solit; 11, Kimalel.

Associations among individuals of the 11 populations revealed by the PCoA calculated based on the Nei’s genetic distance are presented in Figure 4.2. The principal coordinates one and two accounted for 58 % and 21 % of the total variation, respectively. Principal coordinate 1 separated the populations associated with variety *senegal* from those of variety *kerensis*.

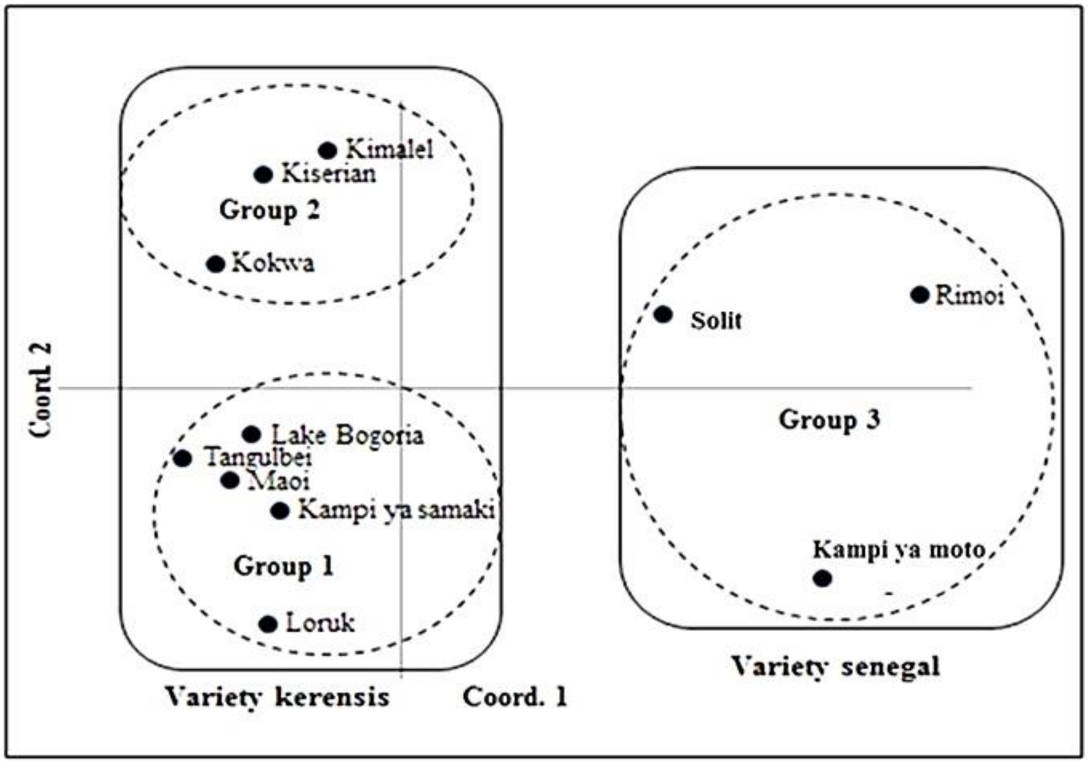

Figure 4.2: Principal coordinate analysis (PCoA) based on 12 microsatellite loci among 11 *S. senegal* populations within Lake Baringo woodland ecosystem.

The topologies of UPGMA dendrogram and the PCoA diagram were identical and consistent with the structure analysis result. The populations were clustered into three groups with two major clusters (Figure 4.2 and 4.3). The first group consists of populations of *S. senegal* believed to belong to the variety *kerensis* while group two cluster with populations believed to belong to the variety *senegal*.

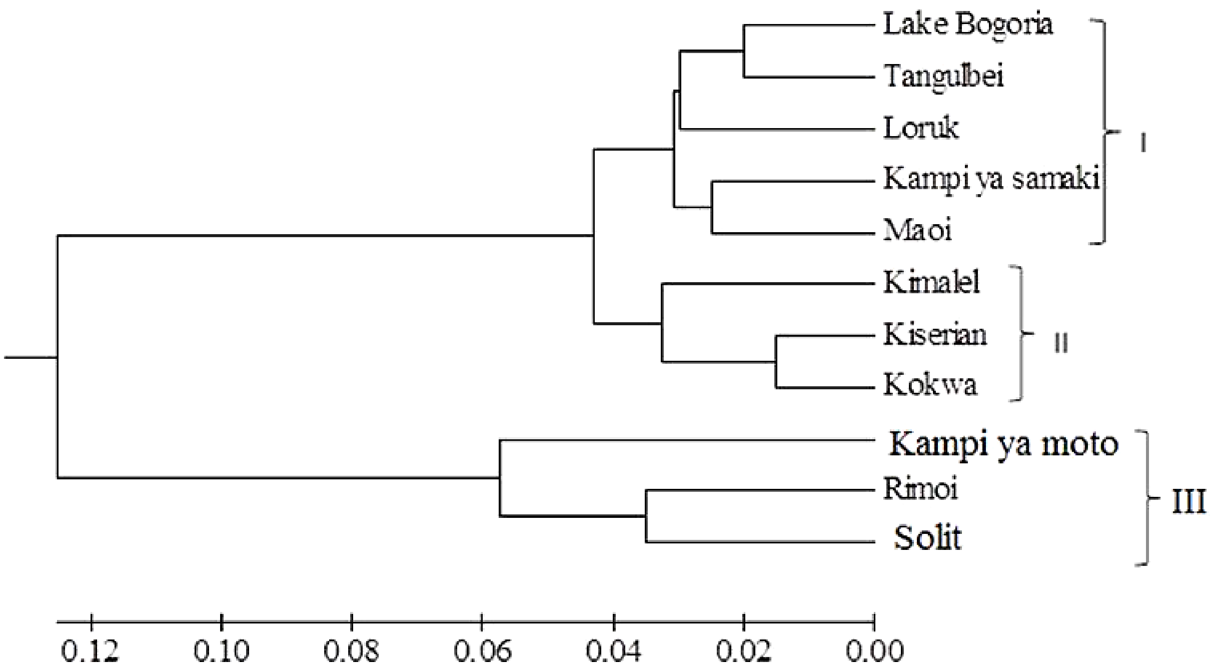

Figure 4.3: Unweighted Pair Group Method with Arithmetic means (UPGMA) dendrogram using Nei's genetic distance for 11 populations of *S. senegal* within Lake Baringo woodland ecosystem.

The average Nei's genetic distance (0.151) was moderate indication of some population divergence within the ecosystem. Correlation between the $F_{ST}/1- F_{ST}$ and the geographical distance matrix for the 55 pairwise comparisons among the 11 populations revealed lack of isolation-by-distance ($R^2 = 0.4262$; $P > 0.05$) among *S. senegal* populations within Lake Baringo ecosystem.

The bottleneck comparison between observed and expected heterozygosities under a mutation-drift model using the Wilcoxon's signed rank test, found Kampi ya Moto population deviating significantly from the mutation-drift equilibrium ($H = 4.14$; $P > 0.05$) under the assumption of the stepwise mutation model (SMM; Table 4.5). No population showed significant deviation from mutation-drift equilibrium under infinite allele model (IAM) and two phase mutation model (TPM). No mode shift was detected in any of the populations under all the models.

Table 4.5: Populations of *S. senegal* within Lake Baringo woodland ecosystem and number of loci with heterozygosity excess (probabilities associated with the Wilcoxon's test in parenthesis)

Population	Model		
	IAM	TPM	SMM
Kiserian	4.58 (0.077)	4.69 (0.285)	4.58 (0.069)
Kokwa	5.24 (0.197)	5.28 (0.447)	5.28 (0.106)
Kampi ya Moto	4.15 (0.147)	4.15 (0.596)	4.14 (0.005*)
Loruk	4.73 (0.097)	4.69 (0.437)	4.69 (0.112)
Kampi ya Samaki	4.63 (0.083)	4.60 (0.211)	4.57 (0.074)
Maoi	5.14 (0.409)	5.21 (0.570)	5.14 (0.135)
Lake Bogoria	4.60 (0.080)	4.65 (0.275)	4.60 (0.214)
Tangulbei	5.27 (0.204)	5.24 (0.438)	5.33 (0.095)
Rimoi	4.71 (0.567)	4.75 (0.053)	4.75 (0.116)
Solit	4.13 (0.398)	4.14 (0.598)	4.08 (0.115)
Kimalel	5.04 (0.615)	5.22 (0.144)	5.18 (0.077)

IAM, Infinite allele model; TPM, two phase mutation model; SMM, simple mutation model was applied *$P < 0.05$

4.4 Discussion

4.4.1 Genetic diversity

Despite the human disturbances and population fragmentations within Lake Baringo woodland ecosystem, genetic diversity of *S. senegal* have remained generally high. However, highly disturbed populations revealed significantly lower genetic diversity values compared to the lightly disturbed populations. The generally high levels of genetic diversity demonstrated by the species may have been promoted by the outcrossing breeding pattern of the species (Obunga, 1995). In such scenario, the breeding system might have been able to offset or weaken the effects of genetic drift (Dai *et al*., 2013). Ideally, the outcrossing and self-incompatibility mechanism may prevent selfing and even to some extent promote long distance gene flow that may sustain high genetic variation. Many tropical species with outcrossing mating patterns have shown similar trend of high levels of genetic diversities (Fernandez-M & Sork, 2007). The mean heterozygosity (H_E = 0.617) revealed in this study is, however, lower than that determined for the same species in the same ecosystem (Marigat, H_E = 0.687) in a previous study involving a range wide sampling within Kenya (Omondi *et al*., 2010b). However, the study by Omondi *et al*. (2010b) did not categorize the populations into disturbance levels. Furthermore, sampling during that study was conducted across the whole ecosystem with the assumption that Lake Baringo woodland was one population. Such sampling strategy may have led to overestimation of genetic diversity of the species. The present study has, however, revealed that Lake Baringo woodland is actually composed of 11 separate populations of *S. senegal* with different genetic diversity levels (Table 4.4). Consequently, the lower diversity values reported in the present study compared to the previous study may have been as a result of the effects of human disturbances that have occurred within the ecosystem over years as reported by Kiage *et al*. (2007). The highly disturbed populations showed lower levels of genetic diversity compared to the lightly disturbed ones which is a sign that the disturbance is creating negative genetic impacts within the ecosystem. The present findings support many previous studies that have suggested that tree species in disturbed natural habitats commonly present lower levels of genetic variation and this may threaten their persistence and evolution (Hamrick & Godt, 1989). But other studies have shown contrary findings with high levels of genetic divergence found across the range disjunctions (Allendorf *et al*., 2013). Such disparities may be attributed to the behavior of agents of gene flow and/or the breeding systems of the individual species.

Comparison of genetic diversity parameters between the two levels of disturbances within the ecosystem reveals moderate but significant differences for allelic richness, mean number of allele and gene diversity. The lightly disturbed populations presented significantly higher genetic diversity, allelic richness and mean number of allele than the heavily disturbed populations. These parameters are usually strongly influenced by species population size and are an indication that density reduction through human disturbances might have acted against these genetic parameters. Human disturbances through selective harvesting or complete clearance act on population by generally reducing densities and population size. The present results concur with those found by Andrianoelina *et al*. (2009) and simulations conducted by Lowe *et al*. (2005). These previous studies suggested that a change normally occurs in trees genetic diversity after human disturbance or fragmentation when the effective population size is significantly reduced. This may actually occur after several generations. The moderate variations reported in the present study could also mean that the human disturbances in this ecosystem are recent and has not shown strong impact. Furthermore, failure to achieve significant difference in F_{IS} values between lightly and heavily disturbed populations may be explained by the species mating system of predominantly outcrossing nature and the presence of self-incompatibility mechanisms (Tandon *et al*., 2001). This is supported by the high values of indirect outcrossing rates reported in the study (Table 4.3).

4.4.2 Population structure and differentiation

Both pairwise F_{ST} and R_{ST} values among the populations revealed moderate but significant genetic differentiation. These results suggest that the species experiences restrained gene flow among the populations as was reported for *Prunus africana* in Ethiopia by Yineger *et al*. (2014). The present results are similar to those reported for other tree species with wider distribution range and similar breeding systems such as *Swietenia macrophylla* or *Caryocar brasiliense* (Novick *et al*., 2003 and Collevatti *et al*., 2003, respectively) and higher than values reported for those species distributed in disturbed and fragmented habitats such as *Grevillea macleayana* (England *et al*., 2002) or distributed in isolated islands such as *Santalum austrocaledonicum* reported by Bottin *et al*. (2005)

or *S. insulare* studied by Lhuillier *et al*. (2006). As seed dispersal in *S. senegal* is sometimes facilitated by animal vectors (zoochory) as was found by Tybirk (1997), possible disturbance and fragmentation effects on agent mobility, abundance and survival might have caused the limitation in seed dispersal in the present study. In particular, the genetic structuring might have resulted from limited spatial patterns of seed dispersal followed by failure of seedling establishment and recruitment filters such as early seedling mortality and selective harvesting of adult recruits (Lacerda *et al*., 2008; Hampe *et al*., 2010).

On top of the possibility of altered gene flow patterns, other demographic characteristics such as differences in effective population sizes, divergence times and colonization history as well as evolutionary processes such as mutation and genetic drift might have also contributed to the genetic differentiation reported here (Hampe *et al*., 2010; Marko & Hart, 2011). Generally, for insect pollinated tree species such as *S. senegal*, low genetic differentiation among populations are normally expected due to the long flight abilities of these pollinators (Beekman & Ratneks, 2000). The moderate differentiation observed in this study may have probably been a combined effect of human disturbances and the presence of two varieties of the species within the ecosystem. Taxonomically, *S. senegal* is differentiated into four varieties (*senegal*, *kerensis*, *leiorhachis* and *rostrata*) of which two (variety *kerensis* and *senegal*) are suggested to occur within Lake Baringo woodland (Brenan, 1983). The presence of the two varieties might have increased the level of population differentiation. To corroborate this hypothesis, the Bayesian approach, PCoA and the UPGMA cluster analyses (Figure 4.1, 4.2 and 4.3) presented a probable separation between the two varieties and confirmed a weak differentiation among populations within these two groups. These results are in concurrence with the expectation of many African *Acacias* and tropical tree species with similar reproductive systems. These species normally maintain more diversity within rather than among populations and/or species (Dayanandan *et al*., 1999).

Strong genetic differentiation may have also been masked by, amongst other factors, the species population size and consequential immense seasonal flowering, presenting a well-synchronized maturity peaks of the flowers (Omondi Pers. com). Mass flowering of *S. senegal* represents the most extreme example of flowering synchrony at both the individual and population levels as was reported for Bignoniaceae family by Gentry (1978). Such synchrony in flowering can influence the levels of gene flow and population differentiations depending on the roles of other agents of gene flow such as pollinators and seed dispersers (Soliva & Widmer, 1999; Domínguez *et al*., 2005). Wokers have shown that, in highly disturbed ecosystems *Apis mellifera* which is known to pollinate *S. senegal*, may expand genetic neighborhood, thereby connecting fragmented populations or isolated trees (Dick *et al.*, 2003). Genetic structure analysis did not show marked isolation by distance of fragmented populations. Therefore, the genetic differentiation reported here is not caused by geographic isolation but most probably the anthropogenic disturbances and varietal differences within the species.

4.4.3 Population bottleneck

There was no sign of severe reduction in effective population sizes of *S. senegal* in the past generations. Given the ongoing deforestation and fragmentation processes, the study might have expected to detect signs of bottlenecks in the remnant populations, especially the more isolated and heavily disturbed ones such as Kampi ya Moto and Rimoi. The present findings, however, may be an additional evidence for the very recent human disturbance and that fragmentation ‘may not yet’ have left a population bottleneck signature in the ecosystem (Dixo *et al*., 2009). Similar conclusion was reported for *Dalbergia monticola* in Madagascar by Andrianoelina *et al*. (2009). The failure of detecting population bottlenecks may also be because of the fact that the power to detect bottlenecks is often low when the decline is not instantaneous or when not many generations have passed since the disturbance (Dixo *et al*., 2009). This shows that the species might have not experienced an instant severe reduction in the effective population size hence still retains high genetic diversity.

In conclusion, anthropogenic disturbances and population fragmentation within Lake Baringo woodland ecosystem has negatively influenced the genetic diversity and population structure of *S. senegal*. The study confirms the hypothesis of presence of low levels of genetic diversity for the heavily disturbed populations compared to the lightly disturbed ones.

CHAPTER FIVE

5.0 EFFECTS OF ANTHROPOGENIC DISTURBANCES ON POPULATION STRUCTURE AND NATURAL REGENERATION PATTERNS OF *Senegalia senegal* WITHIN LAKE BARINGO WOODLAND ECOSYSTEM

5.1 Introduction

Generally, tree species regeneration and population structure respond differently to anthropogenic disturbances. For example, selective harvesting may favour regeneration of some species through reduced competition while others may be negatively affected by being exposed to harsh climatic conditions (Rao *et al*., 1990). Apart from reducing the density of larger trees in the woodlands, anthropogenic disturbances create microclimates by opening canopy gaps which may influence the recruitment and size-class distribution, hence affect regeneration cycle (Brokaw, 1985). Additionally, increased forest disturbances by selective harvesting, livestock and wildlife herbivory may impact negatively or positively on tree recruitment and this may depend largely on the species under consideration and the level of population disturbance (Rao *et al*., 1990; Shackleton *et al*., 1994). In the present study, *S. senegal*, a pioneer and constituent species of *Acacia* dominated arid and semi-arid African savanna woodlands (Fagg & Allison, 2004; Odee *et al*., 2012), was used to determine the effects of anthropogenic disturbance on natural regeneration and population structure. Previous anecdotal observations indicate that the natural regeneration of *S. senegal* appears to have missing growth stages caused by anthropogenic interventions and does not exhibit stable population structure (Omondi *et al*., 2010b). The characteristics of such disturbances often include presence of large herds of grazing livestock, cut

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This chapter has been accepted for publication in the Journal of Forestry Research as “Effects of Anthropogenic Disturbances on Natural Regeneration and Population Structure of Gum Arabic Tree (*Acacia senegal*) in the Woodland of Lake Baringo Ecosystem, Kenya” tree stumps and numerous charcoal kilns distributed across the woodlands. Such extractions may in the long run distort the demography, stability and persistence of the species within the woodland.

The challenges that are brought about by deforestation and degradation of forests may be effectively diverted if focus are geared towards conservation of the remaining forests and restoration of the deforested and degraded ecosystems (Teketay, 1996). There is therefore, urgent need for proper understanding of the dynamics of the species concerned, including causes, mechanisms and factors that influences regeneration process in addition to the population dynamics and recruitments over time. Currently, little knowledge is available about the regenerative capacity and population structure of constituent species within tropical woodlands, especially as impacted by anthropogenic activities. Importantly, regenerative capacity reflects a species ability to establish new seedlings, survive into saplings, and the potential of the saplings to turn into adult trees (Good & Good, 1972). Measurements of these parameters will provide insight into the regenerative capacity of tree species for conservation and sustainable utilization.

The present study was therefore undertaken to determine the density distribution of seedlings, saplings and adult trees of *S. senegal* under different anthropogenic disturbance levels and to examine the size class distribution and natural regeneration status of the species under these anthropogenic levels.

5.2 Materials and methods

5.2.1 Sampling design

Four natural populations namely Kimalel, Kampi ya Samaki, Lake Bogoria and Tangulbei were selected among the 11 populations for this study (Figure 3.1). These populations represented the wider distribution of the species within the ecosystem and the two disturbance levels; heavily (Kampi ya Samaki and Kimalel) and lightly (Lake Bogoria and Tangulbei) disturbed populations (Table 3.1). Inventory was conducted in each of the four population based on systematic sampling design towards the end of the long rains between September and October of 2014.

In each population, 15 temporary sampling plots measuring 400 m^2 (20 × 20 m) were established for the study. Three parallel line transects separated by 100 m were established per population. In each transect line, there were five plots resulting to a total of 15 plots in each population. The first plot was randomly established while the subsequent plots were established systematically after every 300 m within the transect (Figure 5.1). This allowed each plot to be considered as individual sampling unit. A total of 60 sampling units were inventoried in the whole woodland ecosystem.

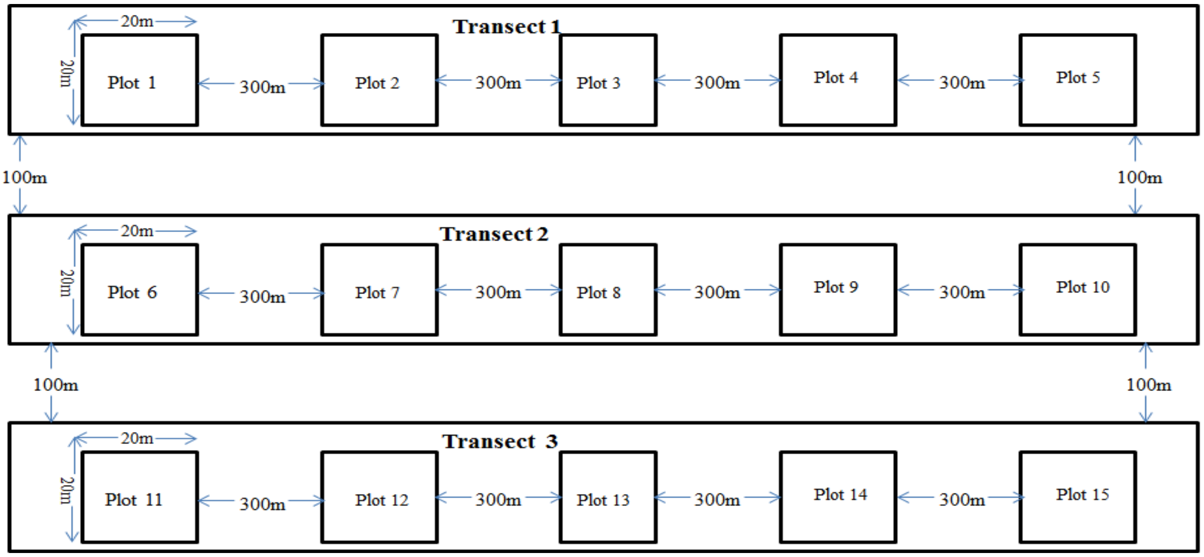

Figure 5.1: Schematic layout of transects and sampling plots for natural regeneration and population structure study within Lake Baringo woodland ecosystem (not drawn to scale).

5.2.2 Inventory, measurements and analysis

At each of the plots established in the four populations, all *S. senegal* individual trees were counted and measured. Tree height and diameter at breast height (dbh) were determined for each tree using extendable meter rule and diameter tape, respectively. The dbh was obtained by measuring the circumference of the tree stem at 1.3 m above the ground. For individual trees with more than one stems, each stem was measured and the final dbh calculated using the formula;

$dq = (\sum_{n=1}^{y} di^2)^{1/2}$, where y is the stems and d_i is the individual stem diameter as described by Gouwakinnou *et al*. (2009). The growth stages of *S. senegal* was determined from the measurements of dbh and height. The height was measured as the vertical length of the tree from the rootcollar to the highest tip. The trees were categorised as follows: adult trees, (> 5 cm dbh), saplings (< 150 cm height and < 5 cm dbh) and seedlings (< 50 cm height) (Traore *et al*., 2008).

Natural regeneration status was determined based on seedlings and saplings population size and by plant renewal rates. Renewal rate was determined by the ratio of regenerates, R (seedlings plus saplings) to M (adults) as R/M. Renewal rate was categorized as follows: R/M < 0 .5, very poor plant renewal; R/M > 0.5 and < 1 poor plant renewal, and R/M > 1 good plant renewal rates as decribed by Traore *et al.* (2008). Density was calculated as the total number of *S. senegal* trees per hectare in each growth stage (Djossa *et al*., 2008). Data on dbh was clustered into eight classes of 2-cm incremental intervals (≤ 2.0, 2.1-4.0, 4.1-6.0, 6.1-8.0, 8.1-10.0, 10.1-12.0, 12.1-14.0 and > 14.0). The size class distributions (SCD) were displayed using bar graphs. Analysis of SCD was done using the method described by Condit *et al*. (1998) and Lykke (1998). One-way analysis of variance (ANOVA) was paformed then followed by Fisher's Least Significance Difference (LSD) model were used to compare means of regeneration and growth parameters between the two-anthropogenic disturbance regimes with significance level set at $P < 0.05$. Spearman's rank correlation analysis was undertaken to evaluate the relationship between adult and regenerates under the anthropogenic disturbance categories. GenStat 16th edition was used for the above analyses (Payne *et al*., 2013).

Structure for each population was determined through estimation of the linear regression slope of the SCD. Ordinary least-squares (OLS) regression was calculated by taking the SCD midpoint (m_i) as being the independent variable. Density at each SCD (N_i) was regarded as the dependent variable. N_i was transformed by ln (N_i+1) to obtain a straight line plot because some size classes had zero individuals (Obiri *et al.,* 2002). The regression analysis was then undertaken using ln (N_i+1) and ln (m_i). The slope of the regression analysis was used to describe the population structure; negative slopes indicate ongoing recruitment (Tabuti, 2007).

Stability of the populations were determined by calculating quotients between successive dbh size-classes. Constant quotients reflected stabile population, whereas varying quotient shows that the population is unstable (Mwavu & Witkowski, 2009). Stability was also determined using other indices such as permutation index (PI) and Simpson's index of dominance (C).

C = 1/N (N-1)$\sum_{i-1}^{k} Ni(Ni-1)$, Where N is the total number of trees and Ni the number of trees in class i, and k is the number of size classes.

PI = $\sum_{i-1}^{k} |ji-i|$ j = 1, 2............k, Where *Ji* is the rank of size class i (*i* = 1 for the smallest trees), with the highest rank (*Ji* = 1) given to the most frequent size class. PI is the sum of the absolute distances between the expected and real location (rank) of all size classes, hence ignoring the relative frequency of different size classes (Wiegand *et al*., 2000).

5.3 Results

5.3.1 Population disturbance index (PDI)

Kampi ya Samaki, Kimalel Tangulbei and Lake Bogoria populations were chosen for this study. The distribution of the PDI in Chapter four indicates that two populations (Kampi ya Samaki and Kimalel with PDI > 40) were more disturbed than Tangulbei and Lake Bogoria (PDI < 40). As a result, Kampi ya Samaki and Kimalel populations were grouped together as ‘heavily disturbed’ while Tangulbei and Lake Bogoria populations as ‘lightly disturbed’. This binary classification is used to assess natural regeneration and population structure differences. In the present study, no control population (undisturbed) was used because Lake Bogoria population which had earlier been thought to be serene, because of being found within a national reserve, also revealed human disturbances hence could not serve the purpose of a control. Due to this anomaly, only two levels of disturbances (light and heavy) are reported and discussed.

5.3.2 Population density and natural regeneration of *S. senegal*

The lightly disturbed populations had the highest mean seedling density; Tangulbei with 1860 ± 503 seedlings/ha and Lake Bogoria with 1696 ± 402, whereas the heavily disturbed population Kampi ya Samaki had the lowest seedling density (Table 5.1). The majority of the seedlings were observed in places that had large canopy gaps and along the fences and paths/roads. Some of these areas had evidence of cut tree stumps. This phenomenon was observed in all the populations studied. Moderate to heavy seedling browsing was indicative of high livestock densities characteristic of the heavily disturbed populations, Kampi ya Samaki and Kimalel (Table 4.1).

The mean seedling and sapling densities were higher in the lightly disturbed populations (Tangulbei and Lake Bogoria) than the heavily disturbed populations (Kimalel and Kampi ya

Samaki). However, based on Fisher's test, only saplings showed significant difference among populations ($F_{3, 56}$ = 5.98; P = 0.001). The sapling densities also revealed a significant difference between light and heavy disturbance categories ($F_{1, 58}$ = 9.50; P = 0.003). Plant renewal rates ranged from 2.02 to 6.43 with the lightly disturbed populations having higher values compared to heavily disturbed ones (Table 5.1). Lake Bogoria population showed a better renewal rate (R/M = 6.43) followed by Tangulbei (R/M = 5.83).

Table 5.1: Seedlings, saplings, adult trees density and renewal rates across four natural populations of *S. senegal* within Lake Baringo woodland ecosytem

		Density (Individuals per ha)			
Population	Disturbance level	Seedlings	Saplings	Adult trees	Renewal
Kampi ya Samaki	Heavy	1402±152a	836±273a	809±121a	2.02
Kimalel	Heavy	1610±318a	597±120a	818±078a	2.70
Lake Bogoria	Light	1696±402a	968±116ab	414±061a	6.43
Tangulbei	Light	1860±503a	1620±432b	597±153a	5.83
DF		3,56	3,56	3,56	
F		2.17	5.98	1.09	
P		> 0.05	< 0.05	> 0.05	

* Mean±SE; Data followed by same letters are not significantly different at 95 % confidence interval based on Fisher's LSD

Both the two lightly disturbed populations (Tangulbei, Lake Bogoria) showed a typical reversed J-shape distribution curve indicative of higher densities in seedlings and saplings than adult trees (Figure 5.2). Both the two heavily disturbed populations deviated from stable structure. Kimalel population revealed a U-shaped distribution curve while Kampi ya Samaki population showed a bell-shaped distribution curve (Figure 5.2).

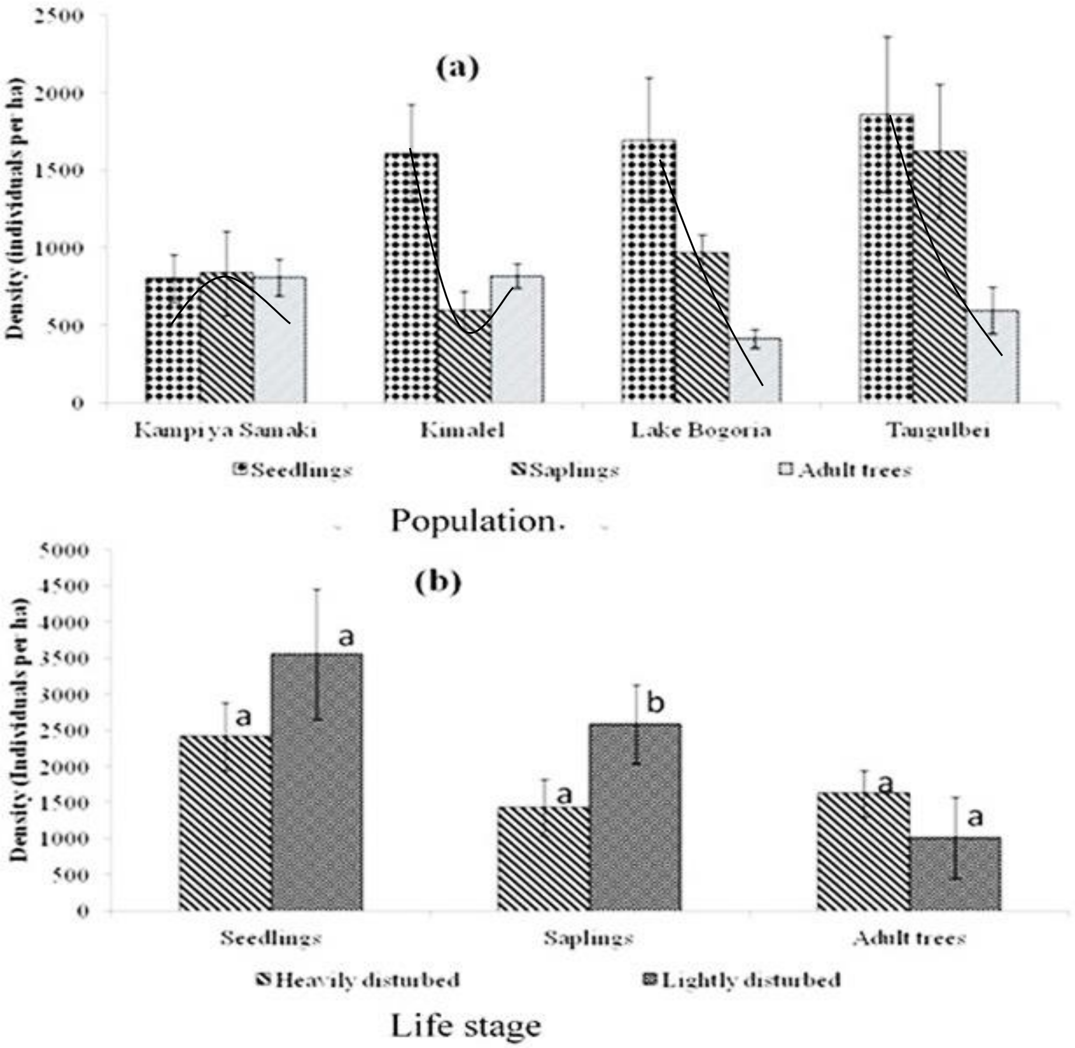

Figure 5.2: Mean population density $(\bar{x} \pm SE)$ of seedlings, saplings and adult trees of *S. senegal* for four populations within Lake Baringo ecosystem. (a) density distribution of life stages per population; (b) summary of life stages density in lightly and heavy disturbances (lower case letters on top of the bars in respective life stages indicate significant differences between the disturbance levels, Fisher's LSD).

5.3.3 Size class distribution and population structure

All the populations had negative dbh SCD slopes. Significant slopes were obtained only for the lightly disturbed populations, Lake Bogoria (R = 0.647; $P < 0.05$) and Tangulbei (R = 0.423; $P < 0.05$) as shown in Table 5.2. Both populations (lightly disturbed) showed a typical reversed J-shaped distribution curve with many trees found in the smaller size-classes compared to the larger classes (Figure 5.3). By contrast, heavily disturbed populations revealed high density of trees in the smaller size-class (dbh ≤ 2.0) and thereafter developed a bell-shaped curve (Figure 5.3). The lightly disturbed populations had most of the large dbh classes missing compared to the heavily disturbed populations. The quotient index was irregular high in heavily disturbed populations indicative of unstable status (Table 5.2). The permutation index results revealed smaller values in the lightly disturbed populations compared to the heavily disturbed populations. However, all the populations deviated from normal distribution with PI > 0.

Table 5.2: Population structure characteristics of *S. senegal* within Lake Baringo woodland ecosystem

Population	Disturbance	Mean dbh	dbh QI	Slope	R^2	*P*-value	C	PI
Kampi ya Samaki	Heavy	5.03±0.12a	2.2	-0.79±0.42	0.386	0.100	0.252	9
Kimalel	Heavy	5.63±0.16a	2.37	-0.88±0.65	0.235	0.223	0.242	11
Lake Bogoria	Light	3.59±0.12b	1.57	-2.38±0.72	0.647	0.016	0.364	4
Tangulbei	Light	4.73±0.09ab	1.73	-1.33±0.91	0.423	0.036	0.321	3

* Mean dbh ± SE; dbh QI, quotient index; C, Simpson's index of dominance; PI, Permutation index. Data followed by same letters are not significantly different at 95 % confidence interval based on Fisher's LSD

Mean quotients determined between successive dbh size classes for all the four populations were irregular showing unevenly distributed population (Figure 5.3).

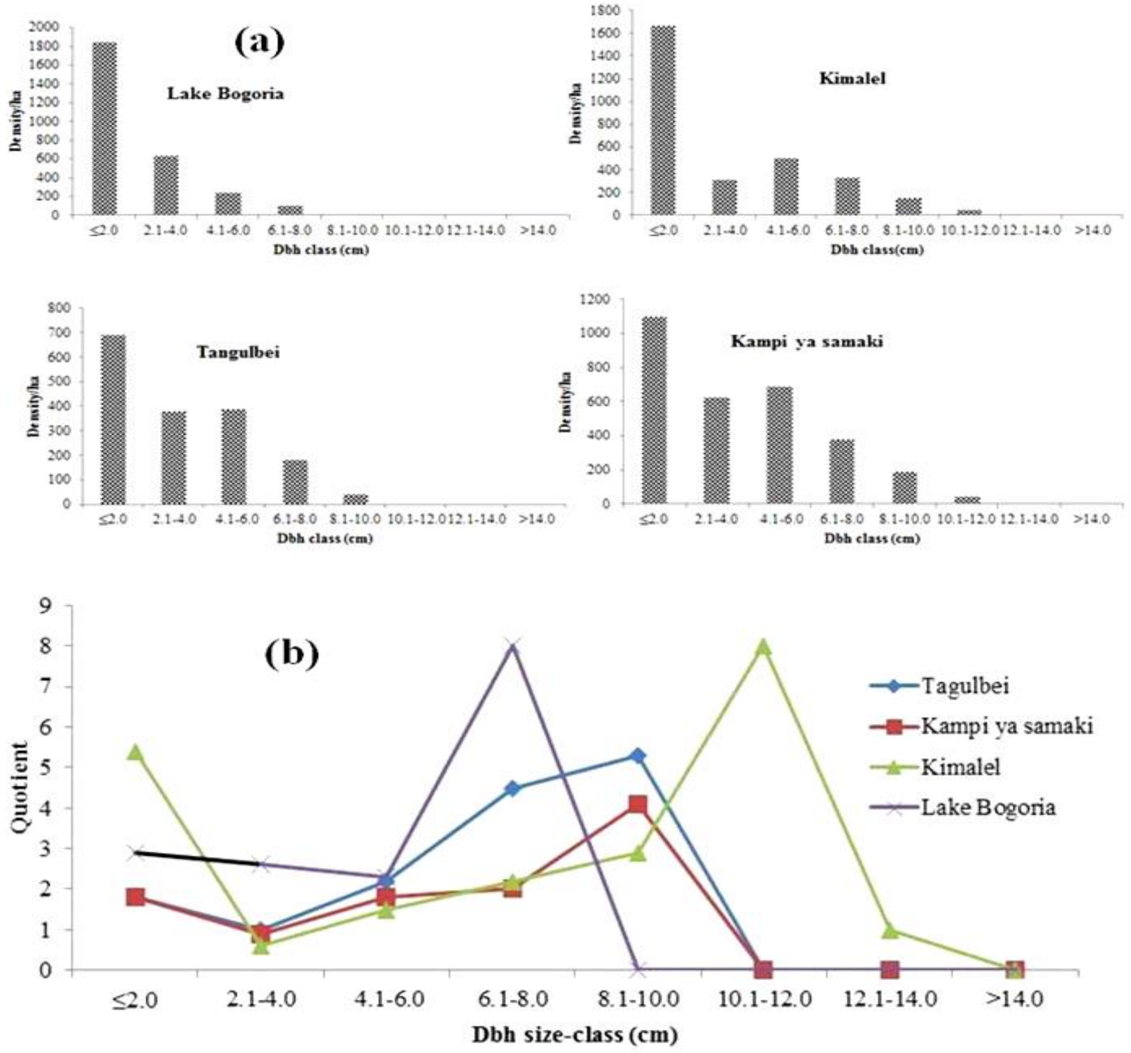

Figure 5.3: (a) Density of different diameter size-classes and (b) Quotients between successive dbh size classes of four natural populations of *S. senegal* within Lake Baringo woodland ecosystem.

5.4 Discussion

5.4.1 Natural regeneration and population densities

Structure of *S. senegal* population as reflected by the quantities of seedlings, saplings and adult trees within the woodland populations varied relative to the levels of anthropogenic disturbance. Generally, the woodland was dominated by high number of seedlings in all the four populations, however, only the lightly disturbed populations exhibited reversed J-shaped distribution curve, a sign of a healthy rejuvenation. During the present study, large numbers of seedlings were observed in open spaces and crown gaps in both lightly and heavily disturbed populations. This indicates the important role played by gaps in natural regeneration of *S. senegal*. As reported in previous studies, *S. senegal* is a pioneer and shade intolerant species and therefore creation of forest gap would enhance its regeneration (Fagg & Allison, 2004). With the availability of sufficient moisture and absence or low livestock browsing, most seedlings of *S. senegal* are found thriving in gaps, abandoned livestock sheds or by the road and path sides as was observed during the present study. Typically, by selectively harvesting adult trees (large diameter trees) for charcoal production or other domestic use, gaps are created that provide favorable micro-climates for seed germination and proliferation of seedlings (Fagg & Allison, 2004). Similar findings were reported by Khan *et al*. (1987) for sub-tropical forests in north-east India where they suggested that removal of overstorey vegetation favors seed germination and seedlings establishment through increased solar radiation and reduced competition from trees of upper canopy.

Higher plant renewal rates were revealed for lightly disturbed than heavily disturbed populations. These findings are clear indication of reduction in regeneration of *S. senegal* with intensification of anthropogenic disturbance. The present results are similar to those reported for protected Acacia woodlands in eastern Burkina Faso by Traore *et al*. (2008) that suggested the presence of negative impacts of anthropogenic disturbances on natural regeneration. Generally, tree population disturbance will definitely reduce the density of a certain growth stage and ultimately distort the population structure. Findings of this study corroborate the results found by Neelo *et al*. (2013) for *Acacia galpini* and *Boscia albitrunca* in Botswana. In the present study, poor regeneration in the heavily disturbed sites may be related to a blend of selective harvesting of adult trees for charcoal production, livestock browsing and clearing land for cultivation.

Declining density trends from seedlings to adult trees are generally regarded as healthy, however, significantly low number of adult trees can also pose serious threat to natural regeneration and evolution of a tree species. Unsustainable removable of adult trees may lead to loss of fitness and narrowing of gene pool for effective seed production and seedling regeneration (Yebeyen, 2006). However, notwithstanding its light disturbance category, Lake Bogoria population recorded abnormally low adult densities. This occurence may be linked to other factors other than anthropogenic disturbances such as soil characteristic that may not be allowing large diameter growths. In this case, even old trees would appear small in size. Based on visual observation, the soils in Lake Bogoria site are rocky and shallow hence may have led to slow or stunted growth of the trees. As a consequence of disturbance, a decrease in adult trees in an area reduces reproductive capacity of the population by hindering cross pollination and lowering seed production (Shackleton *et al.*, 1994). Consequently, loss of sexual reproduction in a tree population could result in poor regeneration and reduced genetic diversity, which may negatively affect long-term persistence of the species (Wilson & Witkowski, 2003). However, the impact of adult tree removal within Lake Baringo woodland ecosystem had not affected the fitness level as evidenced by the large number of seedlings and saplings reported.

Despite the generally high seedling densities of *S. senegal* reported in the woodland, there remains a potential threat from livestock browsing. The leaves of *S. senegal* are highly nutritious (high nitrogen content) and palatable to livestock (Argaw *et al.*, 1999; Fagg & Allison, 2004). Large herds of livestock and signs of heavy seedlings browsing were observed during this study, which is a sign of potential threat to regeneration. Intensive browsing can limit growth and increase plant mortality in heavily disturbed populations. Since local communities within the ecosystem keep large herds of livestock such as goats and sheep that depend heavily on *Acacia* vegetation as source fodder all year round, heavily disturbed populations were characterized by heavily browsed and stunted juveniles (seedlings and saplings). Similar observations have been reported for other *Acacia* species in Burkina Faso and Ethiopia (Argaw, 1999; Traore *et al.*, 2008). In contrast, Kimalel population which is categorized as heavily disturbed, showed high seedling densities compared to the lightly disturbed populations. This suggests that disturbance might have stimulated regeneration by providing light and heat energy to the soil with little mortality realized yet. However, this may change with time as livestock browsing increases and the weather conditions changes (onset of dry spell). Yebeyen (2006) reported similar findings for *S. senegal* seedlings in a disturbed Acacia-commiphora deciduous woodland of Ethiopia's central Rift valley. During the present study, most of the seedlings in this population were mostly found in gaps exhibiting recent selective harvesting. Such extractions stimulate regeneration during rainy seasons in most *Acacia* species since their seeds usually remain dormant for a long time as soil seed bank waiting for a conducive environment (Neelo *et al.*, 2013). Furthermore, it was observed that Kimalel population had high grass cover compared to Kampi ya Samaki. This might have provided sufficient feed for grazers being the rainy season, although, the grass-cover was not quantified during the present study. It is therefore likely that the seedling densities will reduce with the decline in grass cover as the livestock turn to the *Acacia* seedlings for browsing.

As reported above, anthropogenic disturbance exacerbates or impacts key factors that may be essential in sustaining the regeneration process (Brokaw, 1985). However, the low regeneration reported in the heavily disturbed populations may also be seasonal and not permanent, an attribute which is quite common in *Acacia* species (Wiegand *et al.*, 2000; Witkowski & Garner, 2000). Apart from anthropogenic disturbances, it is also likely that these populations could also be periodically affected by water stress due to climate variability and prolonged droughts not uncommon in Lake Baringo woodland ecosystem (Kiage *et al.*, 2007). Furthermore, *S. senegal* regeneration has been previously reported to be significantly affected by moisture scarcity in drier conditions, seed rotting and heavy parasitisation resulting in low seed viability (Otieno *et al.*, 2001).

5.4.2 Size-class distribution and population structure

All the four populations showed signs of adequate recruitment of *S. senegal* recording higher density of trees of low size classes. However, the four populations differed based on level of anthropogenic disturbance as shown by the SCD slopes. Generally, lightly disturbed populations showed gradual reduction in the number of trees from small to larg size-classes while heavily disturbed ones indicated irregular structures.

The lightly disturbed populations showed a reversed J-shaped SCD curve, which indicates that the populations were healthy and rejuvenating. Usually, the stability of a population is reflected in the high numbers of small size-class (juveniles and saplings) which would be recruited into larger size-classes (adult trees) for continuity. On the other hand, the heavily disturbed populations showed a distorted bell-shaped SCD curve (Kimalel and Kampi ya Samaki). In this case, there was high density in the smallest size class (< 2.0 cm dbh) with a rapid drop in the 2.1 - 4.0 cm dbh size class before a rapid upward growth in the 4.1 - 6.0 cm dbh size class. Similar results have been reported for *S. senegal* in Burkina Faso and India by Traore *et al*. (2008) and Yadav & Gupta (2009), respectively. The present results may be as a result of selective harvesting for charcoal production and fencing materials which are common practices within the two populations.

The overall negative and steep SCD slopes, in addition to the size class distribution patterns reported for the lightly disturbed populations (Lake Bogoria and Tangulbei) are indicative of a healthy *S. senegal* populations. This means that there are more juveniles (seedlings and saplings) to sustain the species in the coming generations. The present results are in concurance with the results reported for several other dryland species such as *Balanites aegyptiaca* and *Acacia seyal* in Burkina Faso which showed better regeneration and stable population structure in less disturbed sites than in more disturbed populations (Sop *et al.,* 2010). In comparison with the heavily disturbed populations, the lightly disturbed populations showed steeper negative SCD slopes, an indication of more stable populations that are recruiting successfully. The SCD plots in lightly disturbed populations shows clearly that *S. senegal* has more individual trees in the small dbh size classes wich gradually decreases in the middle size-classes the to large size classes. The present results concur with those reported for *S. senegal* in natural and protected acacia woodlands (low or minimal disturbances) of North Kordofan, Sudan (Eltahir & Wagner, 2012) and Tiogo State Forest, Burkina Faso (Traore *et al.,* 2008). In theory, such trend shows that the populations are healthy or are expanding and are replacing themselves naturally through successful regeneration (Geldenhuys, 1992; Obiri & Lawes, 2000; Sokpon & Biaou, 2002; Tabuti, 2007).

The permutation index (PI) across the woodland reflects the vulnerability of *S. senegal* to anthropogenic disturbance. The lightly disturbed populations showed stable structure by recording low PI values while the heavily disturbed ones reported deviation from a stable population (high PI). The instability was further reflected in the irregular quotient values reported for the successive size classes. However, irregular quotient was reported for both the lightly and heavily disturbed populations. This means that all the four populations are unstable, although, the level differs between the two catergories of disturbances. Generally, fluctuating quotients such as those reported in this study are normally found in savanna tree population patterns, which is often a result of regular habitat disturbance either through fire, vegetation browsing, selective harvesting or climate changes (Shacklerton, 1993). Some of these factors (herbivory and selective harvesting) were observed in the present study sites. The irregular size class distributions reported here may also be linked to episodic recruitment and irregular growth patterns. Wiegand *et al*. (2000) reported a similar pattern for *A. raddiana* populations under anthropogenic disturbance and suggested the influence of episodic recruitment as one of the other contributing factors. Population stability can also be determined via estimation of Simpson's index (C) (Wiegand *et al.*, 2000). A stable population would record a C = 0. All the four populations recorded C values greater than zero. Generally, the slow decline which is represented by a high Simpson's Index would be expected in heavily disturbed populations, high recruitments are usually observed with low density of large size classes targeted by wood harvesters. The present study showed that the heavily disturbed populations of *S. senegal* exhibited higher levels of monotonic decline than the lightly disturbed populations thus corroborating our population assignments to the two disturbance groups.

In conclusion, this study suggests that *S. senegal* natural regeneration and population structure is negatively affected by anthropogenic disturbances. This was evident in the heavily disturbed populations that showed skewed population structure and poor regeneration.

CHAPTER SIX

6.0 VARIATION IN LEAFING, FLOWERING AND FRUITING PHENOLOGY OF *Senegalia senegal* WITHIN LAKE BARINGO WOODLAND ECOSYSTEM

6.1 Introduction

Phenology is often an overlooked aspect of plant ecology, from the scale of individual species to whole ecosystems (Cleland *et al*., 2007). However, phenological studies in plants provide useful information that include growth patterns and developments as well as how these patterns interact with the environment. This will also include the flowering and fruiting dynamics of the plants under selective pressure (Khanduri, 2014). Additionally, flowering in some plants usually signals production time and changing phenological events may also advent of indicate climate change (Zhang *et al*., 2006). Detailed study on the growth of plants and how they develop is a very important step in understanding how the environmental factors affect the life forms in their natural habitats hence able to facilitate conservation efforts. These studies help in the understanding of species ecology, productivity and their contribution to the ecosystems (Duke, 1990).

Timing is a very important strategy in plants, more so during reproduction. This will ensure that the plats flower on time and produce viable seed when the environmental conditions are favorable and there is sufficients resources to sustain the processes (Khanduri, 2014). Poor timing of the reproduction season may lead to poor seed production due to lack of sufficient time for maturation. For example, flowering event which is usually affected by factors such as temperature and photoperiod that are used by plats to signal the onset or end of seasons and are perhaps the well

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understood (Zhang *et al.*, 2006). Proper timing of these environmental cues and how the plant responds to them permits the plant to initiate reproductive phase when the conditions are favorable. In that case, availability of resources and suitable environmental conditions exert selective force on phenological responses.

Senegalia senegal tree flowers at relatively young age, mostly at year three in ideal environments (Fagg & Allison, 2004). The flowering, however, varies between populations but usually occur soon after or just before the begging of the rains (Tandon *et al.*, 2001). This is normally around the time of leaf flush. The flowering pattern of *S. senegal* in East Africa is relatively variable due to the bimodal rainfall with the frequency varying from August/September to January every year (Fagg & Allison, 2004). In some parts of Kenya e.g. Kulamawe and Magadi, the flowering occurs shortly after the rains followed by leaf flush (Obunga, 1995). However, there is limited information on the phenological events of the species in many parts of the country where potential for commercial exploitation and farmland adoption is high. Such information is useful in conservation and sustainable management of the species including utilization in tree improvement (Okullo *et al.*, 2004). Furthermore, disturbed habitats such as Lake Baringo woodland will require conservation and management practices that promote sustainable utilization of *S. senegal* hence the need for knowledge of phenological patterns of the species in this ecosystem.

The aim of the present study was to investigate phenological events in *S. senegal* within Lake Baringo woodland ecosystem in relation to environmental cues at the individuals and populations levels. This involved: (1) investigation, interpretation and documentation of the phenological events; (2) determining the timing of the phenophases; and (3) establishing the relationship between the phenological events of the species with climatic variables.

6.2 Materials and methods

6.2.1 Sampling design

To document the phenological diversity and synchrony/asynchrony within the woodland, 100 m x 100 m temporary sampling plots were established at Kimalel, Kampi ya Samaki, Lake Bogoria and Tangulbei populations. These populations represent the wider species distribution range within Lake Baringo woodland. Documentation was conducted in two consecutive years (25 months, January 2014 to January 2016). Thirty reproductively mature individuals of *S. senegal* trees (> 5 cm girth with presence of previous flowers or pods) per population were selected for this study. The trees were marked with plastic tags and their global positioning system (GPS) data were recorded for ease of identification during the assessments. During the same period, weather data were collected from portable weather stations installed in each of these populations. The weather data collected were; precipitation, soil moisture content, air temperatures and relative humidity. The weather data were then summarized into monthly values.

6.2.2 Phenology assessments

On each of the 30 individual trees selected for this study, four branches per tree, in the north, south, west and east directions were marked for assessments at fortnight intervals. The branches were assessed on one metre length from the tip. During each assessment, leafing, flowering and fruiting processes were scored visually. For leaves; initiation of leaf flush, completion of leaf flush, start of leaf fall, complete leaf fall and the period of leaflessness were recorded. For flowers; flowering initiation, flowering completion, duration between the vegetative phase (start of leaf production) and reproductive phases (when flowers start to appear) were recorded. For fruits; fruiting initiation, completion, initiation of fruit-fall and completion were determined as described by Singh &

Kuswaha (2006). The phenological events were based on the following six stages (0 to 5) as described by Devineau (1999) with some modifications;

(0) No leaf/no flower/no pod

(1) Leaf flush with less than 10 % of new leaves/flower buds with less than 10 % of open flowers/ early pod setting with less than 10 % of mature sized pod.

(2) Leaf flush with 10 to 50 % of new leaves/flower buds present and 10 to 50 % of open flowers/ 10 to 50 % of mature sized pod

(3) Leaves fully developed/peak of flower bloom/peak of pod maturity

(4) 10 to 50 % of the leaves turning brown/peak flower but 10 to 50 % turning dry/ mature pods present but beginning of pod dehiscent and seed scattering

(5) More than 50 % of the leaves dry and leaf fall clearly occurring/more than 50 % of the flowers are dry and the flower fall clearly occurring/more than 50 % of the pods are dry and/or fallen

The totals of the different individuals of the species in each population in the six stages for leafing, flowering and fruiting were calculated for each month.

Phenological events of the populations was summarized by recording occasions separately and for the four populations combined. Various intra and inter population synchrony indices including leaf development, flower formation, and fruit developments were determined as described by Devineau (1999). Synchrony index was determined as the ratio between the mean individual duration of a phenological phase and the overall duration of the phase. The totals of the different individuals of each population for each phenophase, were determined per month. The number of individual trees under each phenophase against the total number of trees under study was used to calculate the precentages per phase. Spearman’s rank correlation analysis was used to determine the correlation between phenological traits with total monthly rainfall, mean maximum and minimum air temperature, soil water content and mean relative humidity.

6.2.3 Floral morphology

Study of the floral morphology followed the protocol described by Nghiem *et al*. (2011) with some modifications. Reproductively mature trees used in the phenology study were also used in this study. The peak flowering season which occurred in May and October were chosen for flower sampling. During this season, 30 flower inflorescence per tree were collected at anthesis and fixed in methanol: acetic acid solution (3:1) for 4 hours. The solution was then replaced by 70 % ethanol. In the laboratory, each inflorescence was measured for length (cm) and the flowers per inflorescence were counted. Each of these flowers were observed under a binocular dissecting microscope and scored for pistil development.

Thirty (30) flowers per inflorescence were randomly selected and their lengths and style lengths measured using dissecting microscope. Sodium hydroxide solution (0.8N NaOH) was used to soften and clear the flowers for about 10 min at 60 °C. The flowers were then stained for 30 min in aniline blue. The flowers were dissected to separate the style from the ovary. The ovary was then divided into two halves. Following the procedures described by Martin (1959), the dissected ovary was viewed under florescence microscope after being placed in a drop of glycerol. This was done to help in counting the number of ovules. Stigma (30) and polyard (30) diameters were measured per tree using light microscope and Axio vision 3.1 software.

Pollen was collected from 30 more inflorescences per tree per population. The inflorescences were dried in a silica gel for about 3 hours for ease of seperation, then sieved through a 63 mm sieve.

Pollen per inflorescence was then grown in a medium containing agar (1 %), sucrose (2 %) and boric acid (0.01 %) at 26 °C. This was basically to determine polyad germination. Germination percentages were determined by viewing the polyard under light microscope after 4 hours' incubation. The presence of at least one pollen tube longer than the polyard diameter was used as an indication of polyard germination.

Thirty pods per tree from the 30 trees selected per population were collected when the pods were mature (brown) and had already started opening up to release seeds. The pods were kept seperately in sampling bag until seed extraction. The extracted seeds were kept separately per pod. Lengths and widths of the pods were measured using a vanier caliper. Seeds per pod were counted and their state of development recorded (undeveloped or fully developed). The developed seeds were measured for lengths and widths using electronic caliper. The mean seed weight for 1000 seed samples was also determined.

Flower inflorescence, flowers, pods and seed parameters were examined using univariate analysis. To test the differences among populations, one-way analyses of variance was performed and their significance tested by use of Fisher's least significance difference (LSD). All data were analyzed using GenStat 16th edition software.

6.3 Results

6.3.1 Environmental cues

During the two years (24 months) of study, there was significant variation in monthly precipitation ranging from 2.3 mm to 118 mm, although, no difference in precipitation was found among the populations. Rainfall distribution mainly followed the typical bimodal pattern, the months with high precipitation being May/June and October/November (Figure 6.1). Overall, the annual total rainfall differed between the two years with the year 2015 recording more rainfall amounts (650 mm) than the year 2014 (582 mm). The mean temperatures varied significantly and followed the rainfall patterns with the rainy months recording lower temperatures than the dry months. Mean daily maximum temperatures ranged between 26 °C and 31 °C and the monthly distributions were as shown in Figure 6.1. The mean monthly minimum temperatures are relatively constant and were found between 15 °C to 20 °C. Mean daily relative humidity for each month ranged from 14.5 to 67 %. It was observed that the higher the monthly rainfall, the higher the mean relative humidity and the lower the mean maximum atmospheric temperatures (Figure 6.1). The soil moisture content was high during the rainy months and low during the dry months. These trends were similar between the two years of the study.

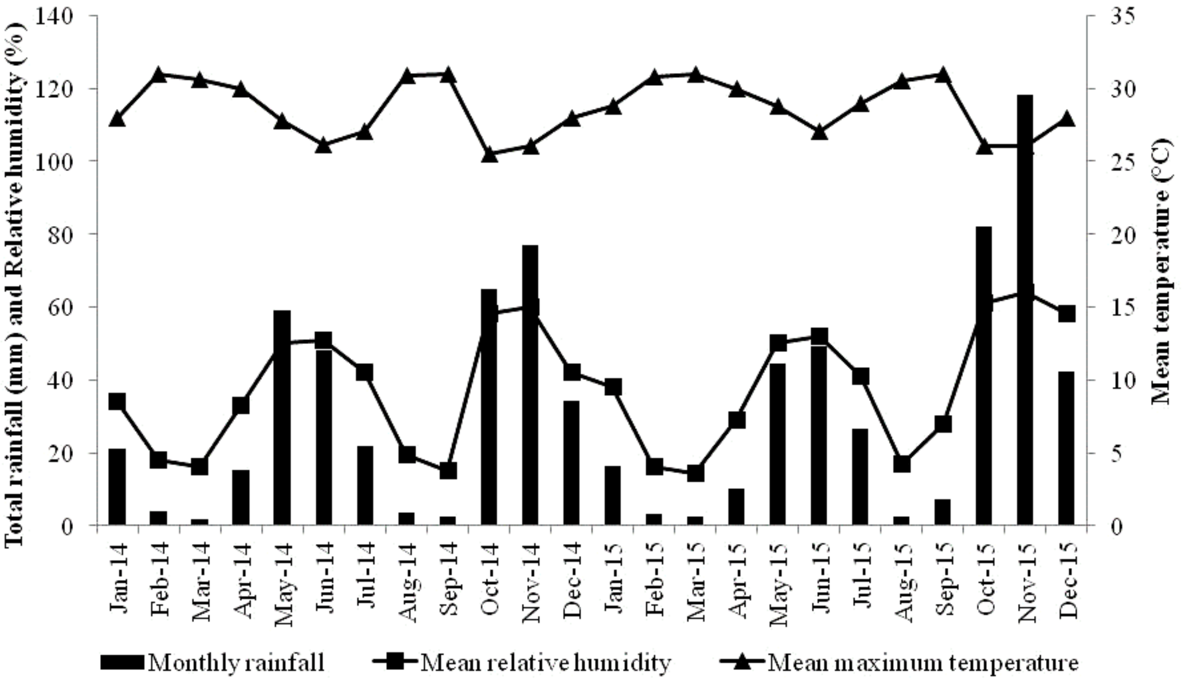

Figure 6.1: Monthly rainfall, mean maximum temperature and mean soil moisture content within Lake Baringo woodland ecosystem.

Records of leafing, flowering and fruiting for the two-year study was made for 120 trees (30 each for Tangulbei, Kampi ya Samaki, Kimalel and Lake Bogoria). In general, all the phenological events were periodic and followed the weather patterns. Due to the two rainy seasons in each year, occurrences of two growth/reproductive seasons per year are evident (Figure 6.2 and Table 6.2).

(a) Monthly phenological events

Population	J	F	M	A	M	J	J	A	S	O	N	D
Tangulbei	♠●	●	○	●♣	●♣♣♠	●♣♠♠	●♠	○	●	●♣	●♣♣♠	●♣♠♠
Kampi ya Samaki	♠●	●	○	●♣	●♣♣♠	●♣♠♠	●♠	○	●	●♣	●♣♣♠	●♣♠♠
Kimalel	♠●	●	○	●♣	●♣♣♠	●♣♠♠	●♠	○	●	●♣	●♣♣♠	●♣♠♠
Lake Bogoria	♠●	●	○	●♣	●♣♣♠	●♣♠♠	●♠	○	●	●♣	●♣♣♠	●♣♠♠

Key
●-Leafing
○-Complete leaf fall
♣-Flowering
♣♣-Peak flowering
♠-Fruiting
♠♠-Peak fruiting

(b) Monthly rainfall data

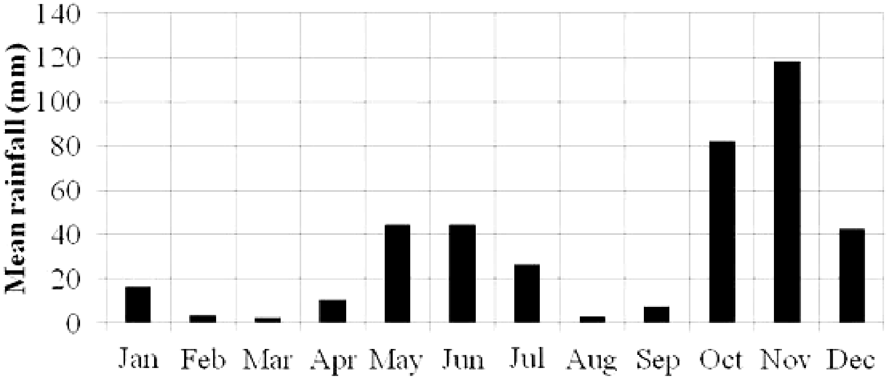

Figure 6.2: (a) Monthly leafing, flowering and fruiting events of *S. senegal* and (b) mean monthly rainfall amounts in Lake Baringo woodland ecosystem.

6.3.2 Leafing phenology

Leaf initiation started with the emergence of leaf buds during the onset of precipitation. This occurred between the last week of September and the first week of October and again in the first week of May for both year 2014 and year 2015 in all the populations. These months correspond to the onset of the short rains of September/October and the long rains of April/May (Figure 6.2 and

Table 6.1). The leaf initiation, however, started one week earlier in Tangulbei and Kampi ya Samaki than in Kimalel and Lake Bogoria populations in both short and long rainy seasons of the year 2014. In the year 2015, the event occurred at the same time for all the populations. The leafing duration (leaf initiation to complete leaf fall) lasted for about 18 weeks for majority of the populations in both the years and seasons but Lake Bogoria population had a shorter leafing duration (16 weeks) than the other populations. The peak of leaf fall (complete leaf fall) occured during the months of March/April and September in both the years 2014 and 2015 coinciding with the hot and dry seasons (Figure 6.2 and Table 6.1). The leaf fall was followed by fresh leaf emergence at the beginning of the subsequent rainy season of May (long rains) and October (short rains). Individual trees did not show any difference in leaf shedding patterns between the years.

Table 6.1: Phenological events of *S. senegal* within Lake Baringo woodland ecosystem for years 2014 and 2015

Year	Phenophase	Population			
		Tangulbei	Kampi ya Samaki	Kimalel	Lake Bogoria
2014	LI	Apr (Sep)	Apr(Sep)	April(Sep)	May(Sep)
	LFI	Aug (Feb)	Aug(Feb)	Oct(Feb)	Oct(Feb)
	PFL	May (Oct)	May(Oct)	May(Oct)	Jun(Oct)
	PFR	Jun (Dec)	Jun(Dec)	Jul(Dec)	Jul(Dec)
2015	LI	May (Sep)	May(Sep)	Jun(Sep)	Jun(Sep)
	LFI	Oct(Feb)	Oct(Feb)	Nov(Feb)	Nov(Feb)
	PFL	Jun(Oct)	Jun(Oct)	Jul(Oct)	Jul(Oct)
	PFR	Jul(Dec)	Jul(Dec)	Aug(Dec)	Aug(Dec)

*LI-Leaf initiation; LFI- leaf fall initiation; PFL-peak flowering month; PFR- peak fruiting month

6.3.3 Flowering phenology

The species had two peak seasons in each year for flowering, which ocurred during the short and long rains. The flowering period in all the populations begun during the start of the rainy season just immediately after the leaf flush. This phase lasted for several weeks (14) with a one-month peak. The flowering occurred between October to November during the short rains and May to June during the long rains. Flowering pattern was similar in both the years, however, the intensity was low during the short rainy seasons compared to the long rains. In the year 2014, the first floral buds were observed at the beginning of May and mid October for Lake Bogoria and Kimalel populations while for Kampi ya Samaki and Tangulbei populations, the first flower buds were observed in the mid of May and mid October. In the year 2015, the flower initiation occurred in the mid of May and beginning of October for all the populations. The peak flowering times was observed in June but extended to the first week of July in 2014 and mid of June to end of July in 2015. This was also observed from the end of October to the beginning of November in both years.

6.3.4 Fruiting phenology

Fruit development proceeded during the rainy season with pod initiation starting in the last week of June and continuing till July while maturation of the pods started mid July and end of August for the year 2014 and 2015, respectively. These periods were not significantly different among the populations despite few day differences. The peak fruiting month, when majority of the individual tree had more than 70 % fruits, was in June/July and December for the year 2014 and in July/August and December for the year 2015 (Table 6.1). The variation between the years was majorly due to variations in the onset of the seasons. For the two years combined, the peak fruiting month occurred in the months of July and December (Figure 6.1). Fruiting phenophase generally lasted for about three months (12 weeks) during the short rains and lasted for about 14 weeks during the long rains in both the years.

6.3.5 Correlation with climatic variable

Spearman's rank correlation analysis between the number of trees in different phenophases and the climatic variables are as shown in Table 6.2. There was positive correlation between leaf initiation and total monthly rainfall (r = 0.358, $P < 0.05$) and mean soil moisture content (r = 0.488, $P = 0.001$). Peak leaf fall was positively correlated to mean maximum temperature (r = 0.414, $P = 0.001$) and negatively related to mean monthly total precipitation (r = -0.618, $P = 0.001$). The correlation between the number of trees flowering during each month (all plots combined) and mean monthly rainfall, showed that flowering occurred mainly in the wet months. This was significant and positively correlated with the monthly rainfall (r = 0.347, $P < 0.05$). There was also a positive and significant relationship between flowering and mean soil moisture content (r = 0.278, $P < 0.05$). The peak fruiting season was positively correlated to mean total monthly precipitation (r = 0.492, $P = 0.001$) and mean monthly soil moisture content (r =0. 398, $P = 0.001$).

Table 6.2: Correlation of *S. senegal* phenological events with climatic factors within Lake Baringo woodland ecosystem

Phenophase		Environmental variables			
		MDT (°C)	MTP (mm)	MRH (%)	MSM (m^3/m^3)
Leaf initiation	r	0.226	0.358	0.116	0.488
	P-value	0.106	0.031*	0.091	0.001**
Peak leaf fall	r	0.414	-0.618	-0.172	-0.322
	P-value	0.001**	0.001**	0.082	0.001**
Peak flowering	r	0.14	0.347	0.121	0.278
	P-value	0.076	0.021*	0.218	0.037*
Peak fruiting	r	0.212	0.492	0.018	0.398
	P-value	0.044*	0.001**	0.912	0.001**

*signicant at $P < 0.05$; **significant at $P < 0.001$; MDT, Maximum daily temperatures; MTP, Monthly total precipitation; MRH, Mean relative humidity; MSM, Mean soil moisture content; r, Correlation coefficient

6.3.6 Synchrony of phenological events

Synchrony of phenological events of individual trees within and among populations is shown in Table 6.3. The leafing stage was synchronous within and among the populations with many individuals initiating leafing phase when the rainy season begins and initiates leaf fall at the beginning of the dry season for both of the years. The overall inter population synchrony ratio for leaf development was 0.87 (Table 6.3). The overall inter population synchrony ratio for flowering and fruiting phenology was 0.75 and 0.85, respectively (Table 6.3). High synchrony ratio indicates coincidence of the phase among individuals or sites.

Table 6.3: Synchrony indices for phenological events of *S. senegal* within Lake Baringo woodland ecosystem

Population	Synchrony index		
	Leafing	Flowering	Fruiting
Tangulbei	0.78	0.85	0.74
Kampi ya Samaki	0.81	0.74	0.88
Kimalel	0.91	0.80	0.79
Lake Bogoria	0.85	0.78	0.82
Overall	**0.87**	**0.75**	**0.85**

*The values are means of the two annual cycles

6.3.7 Floral characterization

The floral characteristics are as shown in Table 6.4. The number of flowers per inflorescence ranged between 89 and 134 with a grand mean of 92.7. The highest mean number of flowers per inflorescence was recorded in Kimalel population compared to the other populations. The least number of flowers was found in Kampi ya Samaki population. Significant difference on the number of flowers per inflorescence was observed among populations ($F_{3,\ 1247} = 23.53$; $P < 0.05$). The inflorescence length ranged from 5.9 cm to 6.2 cm with a grand mean of 6.1 cm. However, no significant difference was found among populations ($P > 0.05$). Significant differences were detected in flower length ($F_{3,\ 1827} = 20.66$; $P < 0.05$), stigma diameter ($F_{3,\ 1827} = 9.19$; $P < 0.05$), style length ($F_{3,\ 1827} = 3.96$; $P < 0.05$) and the mean number of ovules per ovary ($F_{3,\ 1208} = 21.28$; $P < 0.05$) among populations. Generally, considering both years and all the seasons, Kimalel population registered the largest values in flower length, stigma diameter and the mean number of ovule per ovary than the other populations. The longest style length was observed in Kampi ya Samaki population (Table 6.4).

Table 6.4: Floral characteristics of *S. senegal* within Lake Baringo woodland ecosystem

Population	FS	IL	FL	SD	STL	OPV
Kampi ya Samaki	88.99a	6.12a	7.16a	0.24a	6.61b	4.49a
Lake Bogoria	87.68a	5.95a	7.21ab	0.25a	6.63a	4.43a
Tangulbei	89.28a	6.21a	7.33b	0.24a	6.63ab	4.76b
Kimalel	103.21b	6.25a	7.52c	0.26b	6.84ab	5.05c
DF	3,1247	3,1215	3,1826	3,1827	3,1827	3,1208
F	23.53	2.18	20.66	9.19	3.96	21.28
P	0.002	0.274	0.001	0.001	0.001	0.008
Difference between years	ns	ns	ns	ns	ns	ns

*FS, Number of flowers per inflorescence; IL, Inflorescence length (cm); FL, Flower length (mm); SD, stigma diameter (mm); STL, Style length (mm); OPV, Ovules per ovary; ns, not significant; Data followed by same latter are not significantly different at 95% using Fisher's LSD test

6.3.8 Quality of the pollen

Significant difference in polyard diameter was found among the populations with Tangulbei population showing larger polyard diameter compared to the other populations (Table 6.5).

Table 6.5: Stigma, polyard and pollen characteristics of four populations of *S. senegal* within Lake Baringo woodland ecosystem

Population	**PD (mm)**	**SD (mm)**	**PG (%)**
Kimalel	0.165a	0.24a	55.80ab
Kampi ya Samaki	0.173a	0.25a	63.32b
Lake Bogoria	0.175a	0.24a	52.33a
Tangulbei	0.175a	0.26b	60.60ab
DF	3,1196	3,1827	3,472
F	1.84	9.19	4.73
P	0.138	0.001	0.003

*PD, polyard diameter; SD, stigma diameter; PG, pollen germination; Data followed by same latter are not significantly different at 95% using LSD test

Significant difference among populations was revealed for stigma diameter ($F_{3,\ 1827} = 9.19$; $P < 0.05$) with Tangulbei population showing larger stigma diameter than the other populations. In all the populations, stigma diameter was larger than the polyard diameter. Pollen germination was as shown in Figure 6.3. Significant differences in pollen germination percentage was found among populations ($F_{3,\ 472} = 4.73$; $P < 0.05$) with low germination rates observed in Lake Bogoria and Kimalel populations.

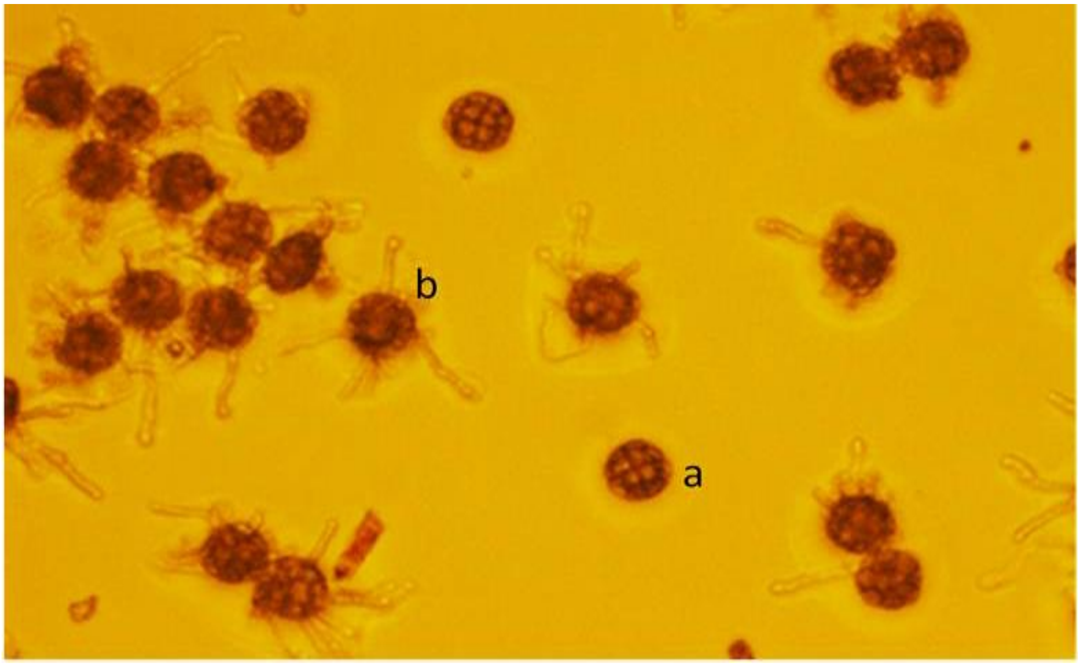

Figure 6.3: (a) Un-germinated and (b) Germinated pollen grains of *S. senegal*.

6.3.9 Seed yield

In both years, the pods measured between 3.4 to 6.9 cm long and 1.1 to 3.2 cm wide (Table 6.6.). Both the pod length ($F_{3,\ 116} = 17.53$; $P < 0.05$) and pod width ($F_{3,\ 116} = 39.39$; $P < 0.05$) were significantly different among populations. Overall, Kampi ya Samaki and Kimalel populations showed larger pod measurements than Tangulbei and Lake Bogoria populations (Table 6.6). The number of seeds per pod ranged between 2 and 6 per population but did not differ significantly among populations. However, most of the trees from Kimalel population revealed more number of seeds per pod compared to the other populations. The average percentage developed seeds per pod per tree were similar in all the populations ranging from 65 to 95 %. No significant difference was realized between years for any of the traits. Significant difference on seed length ($F_{3,\ 116} = 18.97$; $P < 0.05$), width ($F_{3,\ 116} = 28.95$; $P < 0.05$) and weight ($F_{3,\ 116} = 7.10$; $P < 0.05$) was found among populations with Lake Bogoria population showing lower values for both the traits than the other populations. The average seed weight ranged between 42 –76 g/1000 seeds and varied significantly among seasons ($F_{1,\,119} = 12.9$; $P < 0.05$). The long rainy seasons showed heavier seeds per 1000 batch than the short rainy seasons. The weights did not differ significantly between the years.

Table 6.6: Pod and seed characteristics of *S. senegal* within Lake Baringo woodland ecosystem

Population	PL (cm)	PW (cm)	SPP	SW (g)	FF (%)
Tangulbei	4.6ab	2.2b	4a	66b	72a
Kampi ya Samaki	5.2bc	1.8a	5a	71b	81a
Kimalel	5.8c	2.6c	5a	64ab	88a
Lake Bogoria	3.9a	1.8a	3a	58a	93a
DF	3,116	3,116	3,116	3,116	3,98
F	17.53	39.39	2.16	7.10	1.29
P	0.002	0.007	0.137	0.001	0.324

PL, pod length; PW, pod width; SPP, number of seeds per pod; SW, weight of 1000 seeds; FF, fully formed seeds; Data followed by same latter are not significantly different at 95% using Fisher's LSD test

6.4 Discussion

6.4.1 Leafing phenology

Phenological investigations show that in most tropical forests, rainfall is one of the most likely environmental factors controlling the periodicity of tree growth and flowering (Khanduri, 2014). It is also generally believed that occurrence of rainfall after a period of drought or long dry spell usually initiate plant growth mainly in the dry forest ecosystems such as Lake Baringo woodland (Singh & Kushwaha, 2006). The present study has shown that *S. senegal* usually sheds most of its leaves during the dry seasons when the soil moisture content is very low and the atmospheric temperatures are high. As an avoidance mechanism to tolerate drought or the long the dry conditions, the species drop its leaves and regain them during the rainy season (Fagg & Allison, 2004). With the start of the rain seasons, trees produce leaf buds that initiate the leafing phenophase. Such occurrence has been found in many dry forest species (Devineau, 1999). Generally, the onset of rains improves soil moisture content that triggers the tree to begin vegetative growth. Once the first new leaves of the season had expanded, the production of leaf buds and young leaves continued constantly until the whole crown was covered with leaves. During this period, there was no distinguishable transition from old to new leaves. This phase may take between 16 to 18 weeks depending on the length of the rainy season. Complete leaf cover was achieved during the rainy season and correlated positively with high water availability both in terms of precipitation and soil moisture content. This type of development of leaves is more closely related to changing conditions in water availability than was observed for flowering or fruit production as also was reported by Okullo *et al*. (2004). A high percentage of mature leaves are retained almost throughout the rainy season. The leaf formation and duration was observed to be synchronous within and among the four populations. This may be due to the similarity of the dynamics of environmental conditions within the populations. It was noted during the study that, most of the environmental variables occur, generally, at the same time. This triggered the phenological phases of the species in the four populations almost simultaneously. The leafing event was not different between the years. However, some variations were recorded in terms of the length of the phenoevent. This could be explained by variation in the lengths of the climatic variable. For example, the leafless period during the year 2014 was one week longer than that of the year 2015, although, the sequences of events were similar. Similar results have been reported for *Langerstroemia speciosa* by Khanduri (2014).

6.4.2 Flowering phenology

Senegalia senegal flowered during the rainy season which was similar to many other *Acacia* species within the woodland and other tropical species so far studied (Singh & Kushwaha, 2006). During the present study, *S. senegal* flower initiation started few weeks after the beginning of the rains when over 65 % of the crown had been covered by new leaves. The peak flowering was realized at the peak rainy months which signifies the importance of precipitation to *S. senegal* during the flowering season. Most studies have reported that for species that flower's during the rainy season, the onset of heavy rains usually acts as a cue that triggers flowering (Singh & Kushwaha, 2006). During the present study, the peak flowering month positively correlated with the peak rainy months and soil moisture content. Similar finding was reported for *S. senegal* by Tandon *et al*. (2001) in India, indicating that the species prefers flowering during the rainy seasons. It has also been reported that *S. senegal* in some places may respond by flowering even with unseasonal rains (Tybirk, 1993). Principally, most tree species found in dry forest ecosystems normally utilizes the short favorable rainy seasons for leaf development so as to produce and accumulate food reserve and starts the reproductive phase before the soil moisture content reduces in the subsequent drier season (Singh & Kushwaha, 2006).

The interrelationship between leaf formation and flowering/fruiting events in tropical trees species may be attributed to the need for substantial amount of resources to sustain reproduction (Van Schaik *et al*., 1993). *Senegalia senegal* appears to have similar phenological behavior by accumulating leaves for photosynthesise that may sustain it during the reproductive phase. As described by Sing & Kushwaha (2006), production and maintanance of flowers may require large amount of energy for the formation of non-photosynthetic tissues and pollinator rewards required for reproduction success. This phenomenon may require availability of abundant foliage for photosynthesis to sustain the physiological activities during flowering. Some amounts of soil moisture are required during this process hence the rainy season is the best time for the species to flower. The peak flowering month was not significantly correlated with the relative humidity, although, it occurred during high relative humidity ranging between 48 to 62 %. This finding corroborates the results reported by Stone *et al*. (1998), that suggested that relative humidity of between 50 and 60 % is required for efficient pollen transfer. Such high relative humidity may be necessary for *S. senegal* to enhance pollen transfer and fertilization.

The current study revealed significant flowering synchrony among populations and individuals within the populations. The synchrony shows the flexibility of the individual trees that may be useful in maintaining the population and enhance genetic connectivity within the woodland ecosystem. The synchrony may benefit the species by providing an opportunity for pollen transfer within and among populations ensuring high genetic diversity and preventing negative differentiation. Such genetic impact has been reported for the species by harboring high genetic diversity with limited population differentiation (Omondi *et al*., 2010b). The flowering event was similar in both the years in terms of timing and proportion of individual trees with flowers within the months. The two rainy seasons were also not significantly different in flowering intensity. This may mean that the reproductively mature trees flower similarly when triggered by the environmental cues.

6.4.3 Fruiting phenology

The fruiting phase of the species lasted for about three months in both seasons and years. This occurred during the peak rainy season until the seeds were mature and ready for dispersals and probably germination. The fruiting of the species during the rainy season is probably to allow for fruit development and seed maturation since this stage requires a lot of photosynthates (Lieberman, 1982). As the rains subside and the dry season creeps in, almost all the fruits were mature and ripe in readiness for dispersal and germination. Maturation of fruits and presence of suitable environmental conditions for pod and seed dispersal are usually closely synchronized in many tropical dry forest species because of the extreme differences between dry and rainy seasons in these ecosystems (Griz & Machado, 2001).

Senegalia senegal seeds are mainly dispersed by wind and ungulates whose activities are predominant during the dry seasons (Fagg & Allison, 2004). The timing of the season is therefore very important to the species evolution as was also aluded to by Obunga (1995). In most of the dry forest ecosystems, strong winds are common during the dry seasons providing an opportunity for dispersals to wind dispersed seeds like those of *S. Senegal*. Similar results have been reported for a range of wind-dispersed Sudanian savanna woody species in Burkina Faso by Devineau (1999). Furthermore, it is during this period that the *S. senegal* pods are important source of fodder for livestock and other herbivores that are also potential seed dispersers (Fagg & Allison, 2004). In this study, the greater percentage of individuals trees with mature pods (brown pods) were observed towards the end of the rainy season with large number of trees having dry pods occurring during the dry season. The fruiting phenophase was also found to be synchronous within and among populations just as leafing and flowering events. However, the fruits remained longer on the tree in both Kimalel and Kampi ya Samaki populations than in Tangulbei and Lake Bogoria populations. These variations could be attributed to variations in soil characteristics. The soils found in Kimalel and Kampi ya Samaki populations are loamy and therefore able to retain moisture for a longer period compared to the soils of Tangulbei and Lake Bogoria which are majorly sandy and rocky (Sombroek *et al*., 1982). The soil moisture may have sustained the fruits in green form for a longer period in Kimalel and Kampi ya Samaki populations.

6.4.4 Floral morphology and pollen quality

In order to understand *S. senegal* reproductive potential, after every flowering season, the floral morphology and pollen viability were studied. The flowers of *S. senegal* were found to be creamy-white and typically arranged along the inflorescence opening along the axis starting from the base. This observation was similar to the characteristics of this species reported by Fagg & Allison (2004) and Chiveu *et al*. (2009). The floral morphology differed significantly among the populations in all the variables except inflorescence length, although, the variables did not differ significantly between the years and seasons. In addition, analysis of the traits within the populations showed no significant differences. In most of the traits, Kimalel population showed larger values compared to the other populations. For instance, the length of flower inflorescence in Kimalel was the longest and it is from the same population that the largest number of flowers per inflorescence was observed. This indicates that the length of flower inflorescence may have influenced the number of flowers per inflorescence. This is consistent with research reported for some Australian *Acacia* species with similar floral architecture by Nghiem *et al*. (2011). Despite Kimalel having the longest inflorescence length, there was no significant difference on the trait among the populations. The longer inflorescence length and large number of flowers per inflorescence may also be linked to the climatic variability within the ecosystem. This is basically because Kimalel population within the two years received slightly higher amounts of monthly rainfall and retained soil moisture content for longer duration during the flowering period compared to the other populations. Kimalel population also recorded the largest stigma diameter and style length compared to the other populations. The development and growth of these traits may be influenced by the flower size whereby the longer the flower length, the longer the style length and the larger the stigma diameter. Favorable climatic condition may also promote larger sizes of these organs (Nghiem *et al*., 2011).

Generally, pollen quality is one of the very important factors in successful plant reproduction more so to *Acacia* species whose seed production usually occurs after only a single pollination activity (Nghiem *et al*., 2011). Over 50 % pollen germination was reported for all the populations with significant difference among populations. Lake Bogoria population showed lower pollen germination percentage during the year 2014 short rains compared to the other population. This difference may have been brought about by the fluctuation in environmental conditions (rainfall and relative humidity) required for pollen maturity (Kenrick, 1994). During this period, Lake Bogoria population experienced sporadic and shorter rainfall pattern compared to the other populations. This occurrence may have affected the floral maturity by delaying its formation. The flowers might have been caught up by harsh environmental conditions which in the long run affected the pollen quality. Such incidences have been reported by Tandon *et al.* (2001) for other *Acacia* species. However, poor pollen germination may also have been contributed by ageing of the flowers. Flowers collected late after anthesis normally results in poor germination. Probably some of the flowers collected from Lake Bogoria population were old and this might have contributed to the poor germination. Similar results have been reported for *A. mangium* and *A. auriculiformis* and poor germination attributed to both environmental factors and ageing of the flowers (Nghiem *et al*., 2011).

The pollen quality did not differ significantly between the seasons or years. This is contrary to most studies that have shown significant variation between seasons and years (Khanduri, 2014).

However, lack of significant differences within the woodland may be explained by the relatively similar weather condition patterns experienced during the study period. In the long run, the pollen quality reported in this study did not have effect on the number of pods and seed producedper pod. Furthermore, the pollen viability found in all the populations was sufficient to produce open pollinated seeds.

6.4.5 Pods and seed production

Senegalia senegal produced pods with variable dimensions. The pod length ranged from 3.4 to 8.2 cm and the width varied from 1.1 to 3.2 cm. These values were significantly different among populations although, no differences were found between the years or seasons (long and short rainy seasons). The differences may be attributed to soil factors and environmental variables. Kampi ya Samaki and Kimalel populations which recorded larger pod dimensions than the other populations also recorded higher amount of rainfall than the other populations. In this case, rainfall amount could be a factor in pod sizes reported in this study. Nghiem *et al*. (2014) in their study of fruit morphology of *A. mangium* and *A. auriculiformis* also reported significant variation in these traits and attributed the difference to varying environmental conditions. However, genetic constitution of the individual trees concerned may also play a significant role. Similar variation in pod characteristics were also reported on *S. senegal* by Chiveu *et al*. (2009) and these were attributed to both genetic differences and heterogeneity of environmental conditions among the sites.

Significant variation among populations was also found in the weight of 1000 seeds. Heavy seeds were found in Kampi ya Samaki population but no significant difference were realized within populations. The seed weights also did not differ between the seasons and years. In many studies, seed weight has been viewed to mainly represent genetic differences which may be brought about by the adaptation strategy of species. In most cases, some species tend to develop smaller and lighter seeds in drier and harsh environmental conditions compared to those in favorable environments. Similar results were reported for *S. senegal* from different populations with variable environmental conditions by Chiveu *et al*. (2009). The smaller and lighter seeds in drier areas reported in this study are contrary to the findings reported by Chaisurisri *et al*. (1992) who found out that the seed size increases with dryness in Sitka spruce. They suggested that the drier condition forces the trees to store more food in the seed for use during germination and regeneration. However, such adaptations may vary accordingly with species.

In conclusion, *S. senegal* population within Lake Baringo woodland ecosystem has revealed high levels of phenological synchrony within and among the populations. It is also noted that all the phenological phases were bimodal and environmentally triggered. These phenoevensts were not different between the years. Although, there were significant differences in flower length, stigma diameter, style length and ovules per ovary among populations, these differences were only small and did not affect seed production and quality.

CHAPTER SEVEN

7.0 EFFECTS OF ANTHROPOGENIC DISTURBANCES ON MATING SYSTEMS IN *Senegalia senegal*

7.1 Introduction

Mating system is one of the important factors that determine the pattern of genetic structure and evolution in plants (Eckert *et al*., 2010; Millar *et al*., 2014). These factors influence how genetic information is handed to the next generation and, between and among habitats (Alves *et al*., 2003). Importantly, survival of a tree species in the long-term will greatly rely on the ability of its populations to respond to environmental and habitat changes and this is usually related to the amount of genetic variation present in the species (Beardmore, 1983). Ultimately, variation in the mating system directly influences the pattern of genetic diversity dynamics, status of genetic connectivity and population structure (Millar *et al*., 2014). These processes are important to the evolutionary potential of plant populations (Eckert *et al*., 2010). However, mating systems in trees are dynamic and can fluctuate in time and space. Variations in mating systems (outcrossing rates) has been reported among populations and even among individual trees within a single population and between seeding seasons (Lee *et al*., 2000). These variations may be influenced by several factors including human disturbances, mode of pollination, presence of self-incompatibility systems, availability of pollinators and their foraging behavior, flowering synchrony and phenological patterns (Millar *et al*., 2014). Knowledge of mating systems and the scale at which they operate is therefore essential to understanding the genetic structure of plant populations, more so where anthropogenic disturbance has occurred, for consevation (De-Lucas *et al*., 2008).

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Such information would be useful in genetic improvement and conservation efforts (Frankham *et al.,* 2011). Despite the wide distribution of *S. senegal*, its ecological and economic value, several of its natural relict populations are threatened by anthropogenic disturbances. However, it is not known how these interferences affect mating patterns of the species. Studies have shown that many tropical trees species are vulnerable to habitat disturbances and habitat fragmentation because they are naturally found in low densities, have complex self-incompatible mating systems, high outcrossing rates, and show specialized interactions with agents of pollination and seed dispersal (Dick *et al*., 2003; Lowe *et al*., 2005; Ward *et al*., 2005). Additionally, it has been shown that human disturbances negatively affect plant reproduction by distorting activities of the pollinators, pollen installation, fruit set, rates of outcrossing, and the number of sires in fragmented landscapes (Cunningham, 2000; Aguilar *et al*., 2008).

The aim of the present study was to determine the mating systems in *S. senegal* within Lake Baringo woodland ecosystem and to infer how anthropogenic disturbances and habitat fragmentation may affect the reproductive processes of the remaining individuals of the species.

7.2 Materials and methods

7.2.1 Sampling design

Two natural populations of *S. senegal* with contrasting disturbance levels; Kampi ya Moto, heavily disturbed and Lake Bogoria, lightly disturbed, were selected for this study. A tree was considered to be in heavily disturbed habitat when < 100 trees per ha was surrounded by agricultural fields or open grassland and has a PDI >40 %. Population in lightly disturbed habitats consisted of group of individuals of >100 trees per ha surrounded by undisturbed adult trees, had a PDI < 40 % and was located within a national reserve (Lake Bogoria). In each population, a plot measuring 1000 m by 1000 m (100 hectares) was established randomly. Ten mother trees per population were randomly selected within the plots for leaf sampling and seed harvesting. From each mother tree, 40 open pollinated seeds were collected and kept in separated bags with identification tags. In total, 20 moternal trees were selected and 800 seeds harvested from the two populations. The seeds were germinated and planted in sterile soil in polythene pots and the seedlings grown in a greenhouse conditions for one month. Approximately 50 g of leaves were collected form each seedling for DNA isolation. The leaves collected from the moternal trees were also used for DNA isolation. The offspring from a single maternal tree are referred to here as a family or progeny array.

7.2.2 DNA analysis

The DNA was isolated from both the moternal trees and the progeny arrays from the two populations and analysed using 12 microsatellite markers as described in Chapter three of this thesis. The mating system was estimated by multilocus mating system program (MLTR) using mixed mating model (Ritland, 2002). The model is based on the following assumptions (a) each mating event is due to random cross fertilization (with probability, t) or self-fertilization (with probability, S = 1–t); (b) the probability of outcrossing is independent of the maternal genotype; (c) there is homogeneous pollen pool for all the maternal plants; (d) there is no selection between the time of fertilization and the time when the progenies were genotyped; and (e) alleles at different loci segregate independently. Based on these assumptions multilocus outcrossing rate (t_m), single-locus outcrossing rate (t_s), outcrossing rate between related individuals (t_m-t_s), and the correlation of paternity or proportion of full-sibs among outcrossed progeny was calculated using maximum likelihood procedures (Ritland, 1996). The procedure determines how a pair of individuals are related using the genotypic data while assuming that there is a mixture of full-sibs and unrelated pairs. The standard error of the estimated parameters was obtained by 1000 re-sampling bootstraps. The number of pollen sources fertilizing each family or neighborhood size was estimated as described by Ritland (1989). The inbreeding coefficient of maternal parents (*Fm*) was calculated using the MLTR program (Ritland, 2002). The degree of biparental inbreeding was estimated by comparing multilocus (*tm*) and single-locus (*ts*) outcrossing rate values (*tm* - *ts*). The test for random outcrossing was initially conducted by testing allelic frequencies of pollen and ovules for heterogeneity by estimating F_{ST} used as a measure of genetic divergence between allele frequencies of the distinct groups. The coefficient of co-ancestry (Θ) among families within and among populations was obtained using the FSTAT program (Goudet, 2002).

7.3 Results

All individual the progenies from the families of the two populations displayed at least one maternal allele, indicating that there was no seed contamination while the experiment was being set. All the 12 microsatellite loci used in this study were polymorphic and segregated from 8 to 20 alleles, with a total of 122 alleles for the two populations. No exclusive allele was detected either for pollen or for ovules from the crosses in either of the populations. However, estimation of genetic divergence between allele frequencies from ovules and pollen were not homogeneous for 83 % ($P < 0.001$, SE < 0.001) of the loci in Kampi ya Moto population, revealing a deviation from random mating (Appendix 1). Heterogeneity of pollen allele frequencies among maternal genotypes in Kampi ya Moto indicated non-random sampling of pollen pool by each maternal tree.

The t_m and t_s outcrossing rates for the two *S. senegal* populations are shown in Table 7.1. The estimates of the t_m were high for the two populations, however, Kampi ya Moto recorded a value significantly different from 1.000 considering the standard error of the mean (t_m = 0.833; SE = 0.016), suggesting a mixed mating pattern with predominance of outcrossing. The individual multilocus outcrossing rates per progeny were also high in all progenies from the two populations with low variations among the progenies.

Table 7.1: Mating system parameters in two density contrasting populations of *S. Senegal* within Lake Baringo woodland ecosystem

Parameters	**Lake Bogoria**	**Kampi ya Moto**
Number of families	10	10
Number of individuals	102	100
Multilocus outcrossing rate (t_m)	1.000 (0.183)	0.833 (0.016)
Single locus outcrossing rate (t_s)	0.926 (0.079)	0.949 (0.134)
Mating among relatives (t_m - t_s)	0.074 (0.373)	-0.116 (0.171)
Correlation of outcrossing rate (*rt*)	0.07 (0.026)	0.12 (0.057)
Correlation of selfing among loci: *rt*(*l*)	0.936 (0.078)	0.913 (0.106)
Fraction of apparent selfing due to biparental inbreeding: 1 – *rt(l)*	0.064	0.087
Correlation of the estimate of *p* multilocus (*rp*)	0.055 (0.439)	0.329 (0.762)
Probable number of pollinators (*1/rp*)	18.182	3.039
Multilocus parental inbreeding coefficient (F_M)	0.034 (0.081)	0.031 (0.068)
Coefficient of coancestry (Θ)	0.045 (0.097)	0.233 (0.043)

*Standard errors are shown in parentheses

Multilocus outcrossing rates (t_m) was equal to one in Lake Bogoria population (i.e. 100 % outcrossing) while it was significantly lower in Kampi ya Moto population. Mean t_s was lower than t_m for Lake Bogoria population while it was higher than t_m for Kampi ya Moto population (Table 7.1). Consequently, the difference t_m-t_s (biparental inbreeding) was positive and lower for Lake Bogoria population, while it was negative and significantly higher for Kampi ya Moto population (Table 7.1). The fixation index for maternal genotypes (*Fm*) for Lake Bogoria population and within progenies was low and not significant, indicating the absence of inbreeding (Table 7.1). However, *Fm* was significantly different from zero for Kampi ya Moto population, where the density of reproductive individuals was low (Table 7.1).

Correlation of outcrossing rate (*rt*) and correlation of outcrossed paternity within progeny arrays (*rp*) presented varied values for the two populations. For Lake Bogoria population, both the *rt* and *rp* values were low indicating that the outcrossing rate was independent of progeny arrays and that the probability of finding full-sibs within the progenies was very low. For Kampi ya Moto population, the *rp* value was high (*rp* = 0.329) indicating that large number of the progenies from the same array were derived from related crosses, sharing the same paternal and maternal genitors. The low estimate of the correlation of outcrossing (*rt*) in both populations agrees with the low variation observed in the outcrossing rates among progenies within populations. This confirms the outcrossed origin of most of the plants in the progenies. The estimation of the probable number of pollinators (1/*rp*) indicated that about 18 fathers contributed to individual progeny arrays in the Lake Bogoria population while only about 3 fathers contributed to the progeny arrays in Kampi ya Moto population.

The mean co-ancestry coefficient among plants within families of Kampi ya Moto population was close to the expected values from full-sib families ($\Theta = 0.233$; SE = 0.043), and was significantly different from zero, based on standard error. However, coefficient of co-ancestry for Lake Bogoria was low ($\Theta = 0.045$) and not significant showing little possibility of presence of full-sibs.

7.4 Discussion

The 12 microsatellite markers used in this study detected high allelic variation in the populations of *S. senegal* confirming the importance of these markers for the study of mating systems. This is the first report to use such a large set of microsatellite markers to estimate mating systems in African *Acacia* species.

In this study, *S. senegal* displayed a predominantly outcrossing mating system with both the populations recording high levels of multilocus outcrossing rates. Multilocus outcrossing rates estimated for Lake Bogoria population did not differ significantly from complete outcrossing (t_m = 1.000) suggesting that the species may be self-incompatible. However, some mating events occurred among close relatives ($t_m - t_s > 0$). These estimates are in agreement with most of the findings reported for tropical forest tree species (Ward *et al*., 2005). These include species such as *Tachigali versicolor* (t_m = 0.998; Loveless *et al.*, 1998), *Cordia alliodora* (t_m = 0.966; Boshier *et al.*, 1995), *Shorea leprosula* (t_m = 0.837; Lee *et al.*, 2000), *Pterocarpus macrocarpus* (t_m = 0.719-0.959; Liengsiri *et al.*, 1998), *Caryocar brasiliense* (t_m = 1.000; Collevatti *et al.*, 2001), *Acacia auriculiformis* (t_m = 0.863 – 1.059; Khasa *et al*., 1993). The results are also in agreement with the few mating system studies that has been carried out on other *Acacia* species where high outcrossing rates with insignificant levels of selfing having been reported (Khasa *et al*., 1993; Casiva *et al*., 2004).

The mating pattern reported for *S. senegal* in this study can be attributed to its flower development process which may present self-incompatibility mechanisms. All members of the *Senegalia* genus have protoandrious flowers, a mechanism that promote outcrossing, although there can be great outcrossing rate variations among populations (Tandon *et al*., 2001). Generally, self-incompatibility mechanism may be pre-zygotic or post-zygotic. The anatomic structure of *S. senegal* flowers present physical barriers isolating the stigma from anthers ensuring pre-zygotic incompatibility (Obunga, 1995). However, some self-pollen may occasionally be deposited on the stigma but self-incompatibility operating inside the embryo sac may not allow fertilization, presenting post-zygotic incompatibility (Obunga, 1995). Post-zygotic incompatibility mechanisms are evidenced by high abortion of immature seeds following selfing have also been demonstrated in other acacia species such as *Acacia baileyana* (Tandon *et al*., 2001). Such mechanisms are also likely to influence the outcomes of mating patterns in *S. senegal*. These results suggest that *S. senegal* have an efficient self-incompatibility mechanism in place and that selective abortion may increase the outcrossing rate (t_m) by eliminating self-pollinated seeds (Obunga, 1995; Tandon *et al*., 2001). The mating pattern may also have been influenced by pollinators foraging behavior among and within the trees, which is affected by flowering density in the population (Murawski & Hamrick, 1991).

The present findings corroborate the results of controlled pollination study on *S. senegal* by Diallo *et al*. (1997). During that experiment, it was reported that the percentage of flowers setting fruits varied among the pollen source. This included pollen of flowers from the same inflorescence (0.12 %), flowers within the same tree (0.69 %) and flowers from different trees (1.44 %). During the same study, it was realized that natural open pollination produced 1.75 % fruit set. In this case, selfing led to lower fruit set compared to cross pollination demonstrating the importance of outcrossing to *S. senegal* reproduction system. In general, tropical tree species have demonstrated a mixed mating system with the predominance of outbreeding and an outcrossing rate above 85 % (Alves *et al*., 2003). Furthermore, plants with long life span, such as *S. senegal* trees, usually tend to accumulate large somatic mutations and selfing could lead to extinction.

The outcrossing rates of the two *S. senegal* populations were high but significantly different. Variation in outcrossing rates among populations similar to the one reported here has been found in other tree species and probably reflects anthropogenic, genetic and environmental differences among populations (Liengsiri *et al.*, 1998; Coates & Hamley, 1999; Lee *et al.*, 2000). Liengsiri *et al.* (1998) suggested in their study that the variation observed in outcrossing rates among 11 natural populations of *Pterocarpus macrocarpus* may be attributable to the habitat disturbance levels and the density and distribution of flowering trees within the populations. In the present study, Kampi ya Moto population of *S. senegal* which is located in a more disturbed habitat with sparse distribution of trees than Lake Bogoria population, had a significantly low level of outcrossing and showed more biparental inbreeding. This shows that the habitat disturbance might have influenced the level of outcrossing of the species in this population. Kampi ya Moto population was characterized by low density and the trees were scattered in distribution due to human disturbances compared to Lake Bogoria population.

The relatively large variation realized between the multilocus and single locus outcrossing rate estimates (t_m - t_s = 0.116) for the disturbed population (Kampi ya Moto) provides evidence that, despite the high outcrossing rate, a considerable degree of inbreeding of biparental origin (caused by correlated matings) led to the genetic variation of the seed population. The biparental inbreeding suggests a spatial genetic structure among adult trees. This finding agrees with other studies that have found higher levels of biparental inbreeding in disturbed and fragmented habitats compared to continuous populations (Mimura *et al.*, 2009). The biparental inbreeding may be as a result of assortative mating patterns where tighter synchrony in flowering phenology between genetically related individuals is dominant (Lemes *et al.*, 2002).

Another explanation to the high biparental inbreeding reported for Kampi ya Moto may probably be to low adult tree density in the population. The reduction of adult tree densities in the disturbed population might have influenced the pollination dynamics by favouring breeding among relatives. This is phenomenon quite common in sub-structured habitats where random mating occurs within demes, and between closely related individuals (Ritland, 1996). In fact, Kampi ya Moto population is characterized by low density of adult trees hence the likelihood of corroborating the hypothesis of mating between near-neighbour individuals that might be sharing the same lineage. This might have been enhanced by short distance of seed dispersal. *Senegalia senegal* seeds are largely dispersed over short distances by wind, however, long distance by ungulates are also common (Tybirk, 1997). The short distance seed dispersal may ensure that the trees that are found together are likely to be closely related. However, the limited biparental inbreeding in undisturbed population might have been enhanced by the random mating among individuals within the population and sufficient number of available mating partners. This is might have been enhanced by the high density of adult individuals of the species found within Lake Bogoria population.

The correlation of paternity estimate was high (*rp* = 0.329) for Kampi ya Moto than Lake Bogoria population. This suggests that more full-sib offsprings were recorded in Kampi ya Moto population. According to Alves *et al*. (2003), few studies with tropical tree species have reported estimation of correlation of paternity and most of these show higher value for disturbed populations. For example, the *rp* estimation for *Esenbeckia leiocarpa* populations ranged from 0.749 to 0.986 (Seoane *et al.,* 2001). However, correlation of paternity estimates for *S. senegal* is close to ranges reported for *Cariniana legalis* populations, 0.212 to 0.324, and *Tabebuia cassinoides,* 0.295 to 0.547 by Sebbenn *et al.* (2001). The causes for correlated outcrossing in

Kampi ya Moto population might have been brought about by either pollinator behavior, whereby due to sparse distribution of trees, only the neighboring trees are visited and polyandry or deposit of multiple pollen grains from one pollinator (Sun & Ritland, 1998).

The estimated multilocus outcrossing rate of Kampi ya Moto population (0.833), combined with the high paternity correlation (*rp* = 0.329) could be concluded that approximately 27 % (t_m x *rp*) of the offspring were derived from correlated breeding (full-sibs) suggesting that more flowers are pollinated by the same pollen donor in this population. However, the majority, 73 % (1 - *rp*) were derived from random matings, regarded as half-sibs. For Lake Bogoria population, only about 6 % could be categorised as full-sibs. Generally, the increased biparental inbreeding and correlated mating event observed in the disturbed population may be attributed to inbreeding in the adults or spatial population structure (Austerlitz & Smouse, 2001). However, no inbreeding was detected in any of the two populations and therefore spatial distribution of the genotypes may be the important factor in directing the mating pattern realized in this study. Similar findings have also bee reported by Suarez-Gonzalez (2011) in chokecherry species (*Prunus virginiana*).

Density of adult trees may influence the availability of mating partners within a population hence low densities may result in reducing the outcrossing rates as was observed in Kampi ya Moto population. The estimated number of effective pollinators (1/*rp*) was high for Lake Bogoria (more than18) compared to Kampi ya Moto population (about 3). This allowed crossing of many individuals within the undisturbed population unlike disturbed one. As described by Mitton (1992), plant density represents one of the most important demographic factors affecting mating system pattern, as well as extent of the flow of genetic information and level of genetic structuting within and among populations. As the distance between stands and individual trees increases in species with bisexual flowering, generally the higher proportions of the available pollen are from self leading to higher rates of self-fertilization (White *et al*., 2007). This theory cannot hold for *S. senegal* because no selfing was realized in this study. This may be due to the presence of self-incompatibility in the species that prevent maturity of selfed seeds (Obunga, 1995). However, it has been reported that variation in stand density may influence the density of flowering individual trees and ultimately the outcrossing rates (White *et al*., 2007).

The reduced density may also influence the foraging dynamics of pollinators and affect the outcrossing rates. Honey bees frequently visit *S. senegal* flowers and are reported as the potential pollinators of the species (Obunga, 1995). Generally, bees tend to visit more than one flower on the same inflorescence or different inflorescences on the same plant before moving to the neighbouring plants and may come back again to the same flower later (Southwick & Southwick, 1992). Therefore, flight distance of honey bees is commonly short. With the reduced density, interplant distance increases and frequency of interplant flight of bees will decrease, which may lead to increased selfing or near neighbourhood matings. This may explain some of the low outcrossing rates reported in Kampi ya Moto population. Studies have shown a positive correlation between the density of flowering plants and the outcrossing rates such as the case of *Cavanillensia plantanifolia* (Murawski & Hamrick, 1992). This theory may explain the slightly lower outcrossing rate reported for *S. senegal* in Kampi ya Moto population, however, this is not always observed.

Likewise, the estimated value for co-ancestry within families for Kampi ya Moto population (Θ = 0.233 (0.043) was similar to those values expected in full-sib families (0.25) unlike for Lake Bogoria population. Families from open-pollinated crosses as evident in Lake Bogoria population are mostly considered as random mating, resulting in half-sibs. The observed results therefore confirm this hypothesis for high density populations of *S. senegal* and not for Kampi ya Moto. This demonstrates that most families are half-sibs for the dense population and full-sibs for sparse population.

Several factors could account for the reported allelic frequency variations found between the ovules and pollen. This may include nonrandom mating events and pollen immigrating from outside the population (Lee *et al.*, 2000). These results suggest that the two processes might both be important in determining the heterogeneous status of the pollen and ovule allele frequencies. The significant level of biparental inbreeding and high paternity of correlation indicates the occurrence of nonrandom mating, in particular for Kampi ya Moto population. This observation could be explained by immigration of pollen from outside the area sampled, which is consistent with results from estimates of pollen dispersal, where long distance pollen movements was reported.

In conclusion, *S. senegal* has revealed a mixed breeding system with a predominance of outcrosing. However, anthropogenic disturbances reduced the outcrossing rates of the species and promoted mating among relatives.

CHAPTER EIGHT

8.0 EFFECT OF ANTHROPOGENIC DISTURBANCES ON GENE FLOW PATTERNS IN *Senegalia senegal*

8.1 Introduction

Seed and pollen dispersals are the major agents of gene flow in plants and these usually vary greatly among species (Kremer *et al*., 2012). Gene flow, in addition to natural selection, genetic drift and mutation, play significant role in determining the design of genetic arrangement of tree populations (Burczyk *et al*., 2004). Seed and pollen dispersals, in the long run, determines how a species is spreading within a landscape and also controls how genes are interacting within the species range. In this light, pollen and seed movements are key parameters in shaping the dynamics and evolution of plant populations (Hardy, 2009).

Generally, patterns of seed and pollen dispersal and the variation in mating system directly influence species genetic diversity, connectivity and structure (Eckert *et al*., 2010). These factors are key to the evolutionary potential of plant populations. Population genetic theory predicts disruption of genetic connectivity when populations become small and are fragmented or geographically isolated (Jacquemyn *et al*., 2012). Furthermore, loss of allelic diversity via increased levels of genetic drift, is expected to result in population differentiation and loss of genetic diversity (Young *et al*., 1996). In many cases, both biological and physical processes are important factors that significantly influences effective dispersal of propagules (pollen and seed) in tree species. This also includes how the propagules move from one point to another, how the

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viability is maintained during the movement and the chances of fertilization and production of viable seeds which will finally germinate and lead to establishment og seedlings (Kremer *et al*., 2012). These factors in different combinations will determine the dispersal distances which may range from few metres to several kilimeters, generally, following markedly leptokurtic patterns (Kremer *et al*., 2012).

Meta analyses have shown that species in disturbed habitats are generally associated with limited gene flow and overall low genetic diversity, and increased genetic differentiation. This may be a result of the heightened impacts of genetic drift under conditions of limited genetic connectivity and/or selection under a narrow range of environmental conditions (Leimu *et al*., 2006). The long-term impacts of restricted gene flow on species of disturbed habitats with small disjunct populations and geographically restricted ranges may be expected ultimately to be at risk of extinction (Ellstrand & Elam, 1993). Accurate estimation of gene dispersal both by pollen and seed is important in assessing the factors affecting species population fitness and helps in determining the role of sex distribution within the population and other ecological factors in influencing mating patterns (Burczyk *et al*., 2004). This would be useful in identifying important populations for conservation and designing strategies for conservation that will promote genetic connectivity and at the same time prevent genetic introgression.

The present study determined the patterns of gene flow within two distinct populations of *S. senegal* with contrasting plant densities and anthropogenic disturbance levels.

8.2 Materials and methods

8.2.1 Sampling design

To determine the effects of anthropogenic disturbance on realized gene flow patterns in *S. senegal*, the two natural populations with contrasting disturbance levels which were also selected for mating system study, were also used in the present study. Two successive generations (adult and juvenile trees) were investigated within the two populations. To achieve this, one temporary sampling plot measuring 100 × 200 m was established randomly in each of the populations. Leaf samples from all the adult and juvenile *S. senegal* trees within the plots were collected. A total of 152 adult and 186 juvenile trees in Lake Bogoria and 83 adult and 59 juvenile trees in Kampi ya Moto populations were sampled and leaf tissues collected from them. The tissues were taken to the laboratory and DNA isolated from them. The GPS positions of all the sampled trees were recorded.

8.2.2 DNA analysis

The DNA was isolated from all the samples and analysed through PCR using 12 microsatellite markers as described in Chapter three of this thesis. In order to illustrate the power of markers in the parentage analyses, Nei's genetic diversity (H_E) (Nei, 1978), the exclusion probabilities of the first and the second parent (PE1, PE2) and the null alleles frequencies (P_{null}) were computed for adult populations using CERVUS 3.0 software (Kalinowski *et al*., 2007). This study evaluated contemporary gene flow that occurred during the offspring populations establishment using seedling neighborhood model described by Burczyk *et al*. (2006). The method requires data on spatial positions and multilocus genotypes of two successive generations (juveniles and adults), growing in the same plot. Based on that, the probability of the data is modeled in relation to the genetic compatibility between seedlings and potential parents (maternal and paternal) within a specified area, so called neighborhood, which may be treated as a representation of a local population. The basic rationale of this model is that for each seedling, it attempts to find firstly genetically compatible candidate female parents in the neighborhood of that seedling. This is based on the assumption that the establishment of a seedling is preceded by a seed that dispersal from a maternal plant to the place of seedling establishment (and not merely because seed dispersal might be more restricted than pollen dispersal). Then, for each of those candidate (not excluded) female parents within their neighborhoods, there is an attempt to identify genetically compatible candidate male parents (given particular seedling and mother plant genotypes), because mating between male and female individuals is usually preceded by pollen dispersal from male to female. In other words, the model attempts to reconstruct (in reverse order) the gene movement between different life stages (pollen parent, seed parent and seedling). If both actual parents are located in a close vicinity of an established seedling (and close to one another), the model will consider two alternatives: that one individual will be considered as female parent and the other one as a male parent, and vice versa; assigning appropriate transition probabilities (Burczyk *et al*., 2004, 2006). Seedling neighbourhood model provides maximum likelihood estimates of the rate of seed (m_s) and pollen (m_p) immigration into the neighborhood, pollen dispersal effect (β), seed dispersal effect (γ) as well as selfing rate (s) among locally mothered seedlings (Burczyk *et al*., 2006).

8.3 Results

All the 12 microsatellite markers analysed in the studied populations were polymorphic with a total of 146 alleles detected (mean alleles per locus = 12.17, average expected heterozygosity, H_E = 0.815; see also Table 8.1). Each adult individual analysed possessed its own genotype. The cumulative exclusion probability (*EP*) corresponding to the probability to exclude the first and second parent in each of the populations are as shown in Table 8.1. The *EP* values suggest that the loci used in this study were suitable for parentage analysis.

Table 8.1: Genetic diversity of adult *S. senegal* trees from two natural populations within Lake Baringo woodland ecosystem and discrimination power of the set of microsatellite markers

Population	*A*	H_E	P_{null}	*PE1*	*PE2*
Lake Bogoria	77	0.838	0.046	0.9578	0.9951
Kampi ya Moto	69	0.791	-0.001	0.9036	0.9843

**A*- number of alleles; H_E - gene diversity; P_{null} - null allele frequency; *PE1* - first parent exclusion probability; *PE2* - second parent exclusion probability

The level of *S. senegal* seed immigration in both Lake Bogoria and Kampi ya Moto populations were considerably high. About 42 % and 50 % of the seedlings resulted from seed flow over distances > 50 m for Lake Bogoria and Kampi ya Moto populations, respectively (Table 8.2). The 50 m distance is considered the average seed movement in *S. senegal* through wind dispersal (Obunga, 1995). In addition, about 69 % and 62 % of the seedlings with local maternal parents resulted from pollination by distant males (> 50 m) for Lake Bogoria and Kampi ya Moto populations, respectively (Table 8.2). A small percentage (for Lake Bogoria, 0.57 x (1 - 0.6943) x 100 = 17.6 % and for Kampi ya Moto, 0.5 x (1 - 0.6217) x 100 = 18.9 %) of the seedlings in these populations had both parents that are local. Seed dispersal within the local population was moderate (Lake Bogoria γ = -0.0786, Kampi ya Moto γ = -0.0979), which means that large amount of seedlings originated from maternal trees located both nearby and > 50 m for the two populations. Pollen dispersal distances ranged from 382 to 497 m in disturbed population (Kampi ya Moto) and from 96 to 123 m in undisturbed population (Lake Bogoria). Pollen dispersal within neighborhoods followed a negative exponential distribution (Lake Bogoria β = -0.0145 and Kampi ya Moto β = -0.0085) hence less restrictive.

Table 8.2: Estimates of reproductive parameters for two populations of *S. senegal* within Lake Baringo woodland ecosystem

Reproductive parameter	Lake Bogoria	Kampi ya Moto
Seed immigration (m_s)	0.4236 (0.0315)	0.5000 (0.0051)
Pollen immigration (m_p)	0.6943 (0.0472)	0.6217 (0.0016)
Seed dispersal effect (γ)	-0.0786 (0.0124)	-0.0979 (0.0135)
Pollen dispersal effect (β)	-0.0145 (0.0163)	-0.0085 (0.0131)
Seedling sample size	183	59
Multilocus exclusion probability	0.97645	0.94395

*Estimates obtained by applying the seedling neighborhood model and maximum-likelihood estimation procedure (SE in parentheses)

8.4 Discussion

Effective seed and pollen dispersal estimates reported in this study demonstrated relatively similar patterns in the two populations of *S. senegal* despite the differences in disturbance levels. In both populations, more than 40 % of the seedlings developed from immigrating seeds (i.e. originating from maternal trees outside Lake Bogoria and Kampi ya Moto populations). However, slightly lower percentage of immigration was reported for Lake Bogoria (42 %) than for Kampi ya Moto (50 %) population (Table 8.2). This may be attributed to the low density of individual within Kampi ya Moto population hence less potential maternal trees available within the vicinity. Furthermore, the pods of *S. senegal* are papery and contain few seeds that are easily dispersed by wind to far distances when dry (Tybirk, 1997).

Additionally, strong negative relationship between the probability of maternity and the distance between a seedling and a putative maternal tree was found, as might be expected given livestock and ungulate as the other agents of seed dispersal in *S. senegal* (Omondi *et al*., 2010b). Livestock generally feed on *S. senegal* pods together with the seeds during dry seasons and move them to far places during the search for pasture and water. These dispersal agents would allow seeds from far or adjacent stand to migrate to the population. The within-neighborhood average distance between a maternal tree and a seedling was 28.6 m for Kampi ya Moto and 22.1 m for Lake Bogoria, and was almost similar to the mean distance under random dispersal (i.e. 37.3 and 27.9 m, respectively), which means that the majority of seedlings grew further from the mother tree a sign of extensive and effective seed dispersal. These results suggest that only a small percentage of the seedlings in these populations have parents that are local (about 18 %). Similar results were reported for the Scots pine (*Pinus silvestris*) by Burczyk *et al*. (2006).

Parentage analyses were performed under highly conservative conditions to determine pollen movements. However, the study revealed that large fractions (22 %) of paternity remained unresolved for both populations. The relationship between the distance and the probability of mating was significant in both populations. The study shows that *S. senegal* frequently experience reduced pollen flow in remnant trees (disturbed populations) compared to trees in continuous woodland. The average distance between mates within the neighborhood was 20.6 m and 7.3 m in Kampi ya Moto and Lake Bogoria populations, respectively. The spatial isolation and decline of tree density due to human extractions in Kampi ya Moto population clearly reduced pollen sources that would contribute to progeny siring. Although pollen flow in this case may reveal a certain level of stochasticity, the larger average distance between mates observed in Kampi ya Moto population can partly be attributed to low stand density (i.e. larger distances among neighboring individuals and an opened stand structure allowing more space for pollinator movement within the population). Such decline of potential pollen donors is observed, even though, distance of pollen movement is increased in disturbed habitats. The results show clearly that human disturbances and population fragmentation negatively impacts *S. senegal* pollen flow by reducing the density of pollen sources. These results of the present study are similar to those reported for *Ceiba aesculifolia* by Quesada *et al*. (2013).

The present study reveals that over 80 % of the maternal trees in both populations were pollinated by male individuals outside their neighborhoods. Nevertheless, the observed high levels of pollen gene flow are not surprising for *S. senegal*. This species is well known for extensive seed and pollen dispersal (Fagg & Allison, 2004). *Senegalia senegal* is generally pollinated by honey bees, wasps and small butterflies that are can travel long distances from their nest hence able to transfer pollen to far distances (Beekman & Ratnieks, 2000; Fagg & Allison, 2004). Furthermore, these insects are light and their movements can easily be hastened by strong winds that are quite common in the woodland ecosystems. Interestingly, results of this study indicate that seedlings in undisturbed population were less related to each other than were those in disturbed areas. The negative alteration of mating patterns due to anthropogenic disturbances were observed even though the average distance of pollen movement was almost 4 times higher in disturbed population (Kampi ya Moto). This suggests that pollen movement across greater distances does not necessarily imply greater number of sires (Queseda *et al*., 2013). This was evident since the study found that, while isolated trees in disturbed population experienced pollination at higher distances, they received fewer pollen donors.

As also reported in the studies by Kaufman *et al*. (1998) and Schnabel *et al*. (1998), it was observed during the present study that some adult trees displayed significantly greater reproductive success (Table 8.2). It is believed that these individuals are most likely the female parents, because of the small spatial distance between them and their probable offspring. When both parents were determined, one parent was close to the juvenile plant and the other was distant. These observations suggest that pollen comes from long distances, whereas most seed germinated not too far from the maternal plant. Such findings were also reported for *Gleditsia triacanthos* by Bonner *et al*. (1974). It is hypothesized that when nearby neighbors are more closely related than more distant neighbors, selection could act against pollen of nearest neighbors to reduce the proportion of inbred progenies (Schnabel & Hamrick, 1995). Thus, distant or nonrelated neighbors might display an increased reproductive success by pollen flow.

In the present study, pollen dispersal distance was estimated at an average of 450 m and 110 m for trees in disturbed (Kampi ya Moto) and undisturbed (Lake Bogoria) populations, respectively. Such results could be attributed to the nearest neighbor mean distance between trees. Similar effects of spatial structure of reproductive trees on patterns of pollen dispersal have been reported for species such as *Ceiba aesculifolia* (Quesada *et al*., 2013). In the study by Stacy *et al*. (1996), they reported predominance of local mating in populations of clumped reproductive trees, whereas, a reduction in density of reproductive individuals was associated with increase in pollination distances.

On the basis of honey bee behavior, it is predicted that these pollinators of *S. senegal* could fly long distances, dispersing and carrying pollen over long distances from many pollen donors (Beekman & Ratneks, 2000). Results of the present study together with previous findings (Herrerías-Diego *et al*., 2006; Queseda *et al*., 2013), suggest that density and spatial distribution of reproductive trees, the number of flowers per tree and the presence of food resources during a given time of the year are important in determining the movement and foraging patterns of pollinators and the maximum travel distances. Honey bees can travel long distances up to 15 km to arrive at a foraging area (Beekman & Ratneks, 2000). These agents would move pollen from many donors to far distances reducing differentiation of the genetic structure of populations, particularly in disturbed habitats. Looking at *S. senegal* population, generally, the average pollen movement of long distance was detected based on the neighborhood estimates.

The long distance pollen movement results found in this study are similar to those reported by Nason *et al*. (1998) where pollen dispersal between 5.8 to14.2 km was detected in species of the Moraceae family in Panama. Furthermore, similar pollen movements of over 4.5 km have also been reported for *Swietenia humilis*, a species which is pollinated by small butterflies and bees just as *S. senegal* (White *at al*., 2002). Because both maternal and paternal genotypes were unknown, it could not be determined absolutely whether the long-distance gene flow reported for this study is due to seed or pollen dispersion, however, pollen dispersal is the most probable. Similar long distant pollen dispersals (> 1000 m) have been shown in other tropical trees pollinated by bees and wasps (Nason & Hamrick, 1997). In fact, paternity studies in angiosperms have revealed that individuals from outside the study population frequently sire significant portions of the seed crop of the target population (Devlin & Ellstrand, 1990; Gaiotto *et al*., 2003). It is likely that such long gene flow events are more frequent than expected in most tropical tree populations.

In conclusion, the study shows that *S. senegal* pollen and seeds can move over long distances and can effectively prevent or reduce genetic drift and inbreeding. Anthropogenic disturbances did not prevent the species long distance gene flow and actually, the gene flow distance in heavily disturbed population was longer compared to that in lightly disturbed population. This suggests that even small fragments of the species population are valuable in conservation strategies since they can act as stepping stones to genetic connectivity.

CHAPTER NINE

9.0 GENERAL DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

9.1 General discussions

The change of focus on forest utilization from the high rainfall ecosystems to dryland forests and woodland ecosystems has come about with several conservation challenges (Muturi, 2012). Furthermore, these ecosystems are majorly occupied by indigenous species in their natural habitats, which are slow growing. Due to unsustainable demand for forest products, over-exploitation of genetic resources in these ecosystems has been unprecedented and much of the biodiversity are under threat due to over-exploitation and habitat fragmentation. Overall, for sustainable management and conservation of these genetic resources, knowledge of their reproductive systems and population ecology is paramount. For instance, *S. senegal* is a valuable species with products that are traded both locally and internationally with potential of revolutionizing the economy of these dry ecosystems, but the species has been over-exploited and threatened due to lack of basic knowledge of the biological process and the effects that comes with human interferences.

Genetic variation generally has long term evolutionary consequences and are important units in natural selection. These factors are also key to exploitation in the genetic selection and breeding of superior genotypes. Genetic factors are normally affected by human and natural disturbances. The anthropogenic disturbances and population fragmentation within Lake Baringo woodland ecosystem revealed a negative genetic impact on *S. senegal*. Lower genetic indices were found in heavily disturbed populations compared to the lightly disturbed one. Generally, the species showed high genetic diversity and no bottleneck was detected. The negative impacts brought about inbreeding and genetic drift that might have resulted from population bottlenecks are postulated to have been offset by successful gene flow and predominant outcrossed breeding pattern of the species. It has previously been argued that, effects of anthropogenic disturbance may be slow and may only be realized after a number of generations, more so, in long lived species (Aguilar *et al*., 2008). In the present study, it is, however, not precisely known when human disturbances and population fragmentation began within the ecosystem, but living memory and recently documented evidence of degradation (e.g. Kiage *et al*., 2007; Kiage and Liu, 2009) indicate < 50 years ago, i.e. corresponding to one generation for *S. senegal*.

Despite the tolerance of *S. senegal* to harsh climatic conditions, the present study reveals that anthropogenic disturbances have negatively influenced natural regeneration patterns and population structure of *S. senegal* within Lake Baringo woodland ecosystem. Similar impacts have been reported for *S. senegal* in Burkina Faso (Traore *et al*., 2008). The present study showed that the lightly disturbed populations had growth stage densities and size class distributions with typical reversed J-shape curves, representing stable populations that are regenerating successfully over time, while the heavily disturbed populations had irregular distribution curves indicative of poor regeneration and unstable population structure. However, variations were reported among the populations where some size classes were either over- or under-represented. This is an indication of either episodic regeneration patterns or the impacts of extractive activities attributed to various anthropogenic disturbances. Among the key anthropogenic disturbances found during this study was selective harvesting for charcoal production and livestock grazing. These activities are known to negatively affect regeneration through suppression and lack of seed producers. The trend reported in the present study characterizes a species that is vulnerable to anthropogenic disturbance. This study, therefore contributed useful data that would be essential in developing sound management practices to foster sustainable management and utilization of *S. senegal* in the African dry woodland ecosystems.

Generally, biology of reproduction is majorly applied in evolutionary and systematic studies, however, they are also important for effective conservation strategies in important species threatened by human interference such as *S. senegal*. Seed production, dispersal and seedling establishment are some of the most important stages in the life cycle of a plant (Singh & Kushwaha, 2006; Seifert & Muller-Starck, 2009). Understanding these phases are key to genetic improvement and conservation.

Despite the fragmentation of *S. senegal* population within Lake Baringo woodland and the presence of more or less distinct stands, the species has showed high degree of phenological synchrony within and among the populations. This phenomenon is valuable in promoting crossbreeding and habitat connectivity. It is also noted that all the phenological phases were environmentally triggered and therefore the synchrony reported here may be attributed to almost similar environmental conditions that prevailed within the woodland during this study. The synchrony may also be an evolutionary strategy of the species to sustain reproduction. Furthermore, the seasonal leafing, flowering and fruiting, with associations with leafing and leafless durations, observed in the species suggest reproductive and survival strategies evolved by the species to adapt to the harsh environment (Tandon *et al*., 2001). Although floral morphological traits namely; flower length, stigma diameter, style length and ovules per ovary were significantly different among populations, they were less marked and did not appear to affect crossing among individuals and populations. It is therefore worth being concluded that there was no isolation in phenological events or floral morphology that would prevent inter population reproduction. Such inter population reproduction are important in maintaining high genetic diversity and connectivity among populations.

Owing to its pollination system that is undertaken by generalist pollinators and the presence of some level of tolerance for bi-parental inbreeding, *S. senegal* appears very resilient to habitat disturbances, setting fruit and seed even at low densities of adult trees. The species showed a generally outcrossing mating pattern corroborating the long held notion that *S. senegal* is predominantly an outcrossing species. However, some level of biparental inbreeding was also reported in the heavily disturbed habitats. The predominantly outbreeding mating system found in this study corroborates the conclusion by Obunga (1995) that *S. senegal* is self-incompatible and have comparatively high reproductive output. The self-incompatibility that is predominant in the species is largely related to natural selection geared towards maintain high genetic diversity (Millar *et al.*, 2014). Self-incompatibility is actually regarded as one of the most efficient strategies of preventing self-pollination and ultimately putting on check inbreeding depression in higher plants (Jacquemyn *et al.*, 2012). However, the biparental inbreeding reported for the heavily disturbed habitats may have been caused by low density and lack of crossing mates. This might also been an indication that most of the individual trees within these disturbed populations originated from few closely related parents.

With the present study of gene flow, it appears that *S. senegal* has a flexible pollination system and that human disturbances have not significantly disrupted pollen movement among remnants trees or population. The study showed clearly that *S. senegal* pollen and seeds can disperse over long distances than may have been expected for disturbed tree populations. These may sometimes be much higher than expected for naturally undisturbed populations. Similar conclusion was also reached by Trapnell & Hamrick (2005) on their study of orchid populations. In the present study, *S. senegal* has shown a high level of pollen gene flow that are adequate to prevent or reduce inbreeding and the effects that would be attributed to genetic drift. These findings suggest that even small population fragments of the species can have adequate conservation values as important bridges that would be useful in maintaining the genetic diversity and connectivity among isolated populations (Nason &Hamrick, 1997; White *et al*., 2002). Additionally, the presence of pollinators and their behaviors are important determinant of pollen gene flow among isolated plant population fragments and as pointed out by White *et al*. (2002), all the agents of pollination have a flight distance limit they can move. In that case, the level to which population fragmentation and isolation influences plant species will differ depending on the individual species (Aldrich & Hamrick, 1998). Although the genetic impacts of disturbances are complex and normally varies considerably among species, the present study has undoubtedly proven that relic populations and isolated trees of *S. senegal* may provide useful safeguard to the harmful genetic consequences of anthropogenic disturbances and may be relevant to the long-term sustainability of the species. In this case, many of the remaining trees in disturbed areas and relict fragments may persist as reproductively viable individuals and would therefore be very important both for future natural and artificial regeneration and for population recovery and genetic conservation programs.

9.2 Conclusions

Anthropogenic disturbances and habitat fragmentation negatively affected the genetic diversity and structure of *S. senegal* within Lake Baringo woodland. The heavily disturbed populations showed low genetic diversity indices.

Senegalia senegal natural regeneration and population structure is prone to anthropogenic disturbances. Heavy disturbance leads to low natural regeneration rates and unstable population structure of the species. This study established that selective harvesting and livestock browsing are the major anthropogenic factors affecting natural regeneration and population structure of the species.

Within Lake Baringo woodland ecosystem, *S. senegal* flowers and produces seeds twice a year following the rainfall patterns. This is an indication that the phenological events are significantly correlated with environmental cues. The phenology is synchronous within the woodland and did not vary significantly between the seasons or years.

Senegalia senegal is predominantly outcrossing, however, anthropogenic disturbances within Lake Baringo ecosystem promoted biparental inbreeding and resulted in low outcrossing rates compared to undisturbed populations. This is an indication that habitat disturbances can distort the breeding patterns of the species.

Senegalia senegal revealed a long distance gene flow and habitat disturbances or fragmentation did not significantly hinder gene movement. This may have been contributed by long pollinator flights moving pollen to long distances. Additionally, the long distance gene movement may possibly have been due to its generalist pollination system and the resilient foraging behavior of its pollinators in response to changes in landscape structure and the self-incompatibility nature of the species. Remnant individual in between populations may become useful connectivity facets for the species to maintain decent among populations.

9.3 Recommendations

- Since the current study indicates that disturbance of the species habitat affect the persistence of the species, a sustainable management strategy which involves the local communities is required to promote utilization of the species in a manner that will also conserve it for future generations. Furthermore, it is necessary to educate the local communities on the importance of the species and show them how it can be used to improve their livelihoods sustainably. In areas where selective harvesting is happening due to permission given by the government to produce prosopis charcoal, close supervision is required to ensure that non-target species are not harvested.
- For forest managers and tree breeders interested in seed collection within the ecosystem, the best time to collect seeds should be during the months of February/March and September when the pods have matured and turned brown. However, this can be monitored closely using the weather patterns.
- Further studies are necessary to enhance understanding on how the anthropogenic factors interplay with environmental factors (e.g. soil, climate, geology, shades and gaps) in influencing the regeneration patterns and population structure of the acacia woodlands. Similarly, study under several seasons would be required to shade more light on the impact of anthropogenic disturbance on regeneration patterns.
- Study of the next generations (i.e. young cohorts) may be necessary to provide clear position and patterns of the genetic consequences of habitat disturbance in the populations studied. DNA-based studies to determine the contemporary patterns of genetic variations and population structure such as fine-scale spatial genetic studies may also provide additional information to enhance the present results.

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APPENDICES

Appendix 1: Maximum likelihood estimates and significance for chi-square (χ^2) tests of homogeneity between pollen and ovule frequency of the most common allele for two populations of *Senegalia Senegal* within Lake Baringo woodland ecosystem

Population	Locus	Number of allele	Pollen frequency	Ovule frequency	χ^2
Lake Bogoria	mAsCIRH09	16	0.168	0.381	49.97
	mAsCIRE10	07	0.208	0.364	52.35
	mAsCIRE07	05	0.318	0.286	59.19
	mAsCIRH01	23	0.301	0.286	53.16
	mAsCIRF03	27	0.120	0.286	49.16
	mAsCIRB09	05	0.254	0.182	63.6
	mAsCIRE06	18	0.309	0.150	53.63
	mAsCIRB10	15	0.740	0.792	62.28
	mAsCIRC07	20	0.208	0.304	64.41
	Ab26	09	0.249	0.182	21.44
	Ame03	11	0.276	0.333	83.38
	Ame07	08	0.392	0.278	76.99
Kampi ya Moto	mAsCIRH09	12	0.406	0.294	23.59
	mAsCIRE10	09	0.233	0.294	38.22*
	mAsCIRE07	05	0.229	0.188	40.69*
	mAsCIRH01	17	0.245	0.222	53.73*
	mAsCIRF03	21	0.286	0.235	65.18*
	mAsCIRB09	08	0.254	0.211	92.95*
	mAsCIRE06	14	0.312	0.278	37.03*
	mAsCIRB10	16	0.114	0.444	24.38
	mAsCIRC07	18	0.140	0.409	77.65*
	Ab26	07	0.238	0.211	21.44*
	Ame03	11	0.304	0.263	83.38*
	Ame07	09	0.180	0.333	76.99*

* Indicates significance at $P < 0.01$.