A SYSTEMATIC ASSESSMENT OF THE RELATIONSHIP BETWEEN ALOE MACROCARPA TODARO AND ALOE LATERITIA ENGLER (ALOACEAE).

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES OF ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT FOR THE DEGREE OF MASTER OF SCIENCE IN DRYLAND BIODIVERSITY IN THE DEPARTMENT OF BIOLOGY.

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BY

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DEDICATION

To our late son, Ted Simiyu who will always remain a source of strength and inspiration to his parents.

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ABSTRACT

Species delimitation in the genus *Aloe* has proved complicated due to interlocked morphologies. This study investigated the taxonomic relationships of *Aloe macrocarpa* Todaro and *A. lateritia* Engler using both morphological and molecular techniques. The morphological results indicated that the nature of variation in the populations of the two taxa is continuous. Microscopic studies of pollen grains and the leaf cuticle showed little differentiation among populations of the two taxa. The low genetic distances (<0.3) between the populations studied are in support of the hypothesis that the populations may be conspecific, suggesting infraspecific rank(s) for the taxa.

hinged on the recognition of gaps or character discontinuities. To-date, this view is the backbone of taxonomy, upon which the taxonomic practice draws (Heywood, 1998).

Following the Darwinian Revolution, the validity of the Typological species concept was extensively questioned. A plethora of species concepts were advanced all in the effort to base the taxonomic unit on the processes that led to their recognition. Heywood (1998) records that up to 25 species concepts have been postulated to-date, eight of which have been widely used. These include the phenetic, biological, taxonomic, cladistic, pluralistic, phylogenetic, genetic and evolutionary species concepts.

Different schools of thought were responsible for the various species concepts that were advanced. For instance, the biological species concepts (BSC) resulted from wider recognition by naturalists, geneticists and evolutionists that species occur as reproductively isolated natural entities in the field. Thus the BSC recognizes as separate, species that may show no clear morphological differentiation as long as they are reproductively isolated. Although many taxonomists recognize the BSC, critiques of this concept argue that the BSC only applies to bi-parental sexually reproducing organisms (Claridge, *et al.* 1997).

To most taxonomists (Heywood, 1998), species should be seen as human-made groupings, whose importance to science is operational. On this basis the focus of any species definition should be on the detection and description of recognizable patterns and not in the explanation of the forces of speciation. Essentially, species definitions should be pragmatic and must enable effective division of organisms into units that are useful to recognize, bearing in mind the needs of various user groups.

1.0.1 The Taxonomic species

This concept emerged in appreciation of the fact that species were more than types i.e. that species are made up of natural populations of individuals. Later, it was also recognized that geographical coherence existed between populations in addition to morphological and breeding behaviour. Thus the taxonomic species concept makes use of the morphological-geographical approach.

As defined by Du Rietz (1930) (cited in Hedberg, 1957), the species is defined as "the smallest natural populations permanently separated from each other by a distinct discontinuity in the series of biotypes". This discontinuity is usually measured from various evidences including anatomy, chemistry, breeding behaviour, ecology and cytogenetics. The main convention in this approach is that species delimitation should be based on certain character correlations involving at least one key character even though all others are taken into account (Gornall, 1997).

Hedberg (1957) noted that in sexually reproducing organisms, a species is a system consisting of one or more genetically, morphologically different kinds of organisms that possess an essential continuity maintained by the similarity of genes or the more or less free interchange of genes between its members. Species are thus separated from each other by gaps of genetic discontinuity in morphological and physiological characteristics that are maintained by the absence or rarity of gene interchange between members of different species. When species are regarded as dynamic and changing population entities, one expects to encounter many cases where differentiation has not yet resulted into complete speciation.

1.0.2 Infra-specific variation

Infra-specific variation is frequently encountered among morphological species complexes. It is usually detected by detailed analysis that includes the evaluation of variation within and between populations. As noted by Gornall (1997) in distantly related species, the differentiation within populations is consistently much less than between them. In morphological terms, variation within species is displayed in characters like leaf size and inflorescence branching that could be interpreted as being adaptive. These are seen as evidence of ecotypic differentiation. Conversely, sets of characters with less adaptive capacity are used for distinguishing species.

The most commonly recognized infraspecific ranks are subspecies, varieties and forms. Whereas genera and species are regarded as the work of nature, infraspecific taxa viewed as being accidental products of environmental influence. In modern terms, the subspecies represents 'major' morphological variations (that are known to breed true) while the variety is used for 'minor' morphological variations (Stuessy, 1994).

Since proper delimitation of taxa is fundamental to biodiversity, concerted efforts are required in order to unravel the various species complexes found in nature. This calls for multidisciplinary methodology that combines genetic, morphological and ecological criteria. It should further be emphasized that taxonomic distinction should be made not from differences between single specimens but from differences between groups of specimens (Hedberg, 1957).

1.1 Background

Aloaceae is a relatively large family of monocotyledons with six genera and is composed of succulent-leaved rosulate plants (Smith, 1993). The genera included are *Aloe* L., *Gasteria* Duval, *Haworthia* Duval, *Chortilirion* A. Berger, *Poellnitzia* Uitewaal and *Astroloba* Uitewaal (Smith & Van Wyk, 1992). About 550 species have been described in the family with the main centre of distribution in Southern Africa (Steyn & Smith, 1998). Of the six genera described, only the genus *Aloe* is widely distributed out of southern Africa. *Chortolirion* and *Haworthia* occur to the north of the Limpopo River to a very limited extent.

The generic name *Aloe* was first published by Linnaeus (1753). Different views have been proposed with regard to the etymology of the word 'aloe'. In some instances, it has been equated to the Arabic 'alloch' as given by Linnaeus for species used medicinally. Recent suggestion points to the fact that the name is adopted from the Greek 'alloë' that also referred to the dried juice of aloe leaves from earlier Hebrew folklore (Smith, 1993).

Aloe is the largest genus in the family and has the most diverse morphology. Reynolds (1966) had recorded only 3 species from Socotra, 17 in Arabia and 133 species from the African mainland, north of the Limpopo River. At present, about 80 species are known to occur in East Africa (Carter, 1994), 38 species have been recorded for the Flora of Ethiopia and Eritrea (Sebsebe & Gilbert, 1997). Lavranos (1995) recorded 31 species for the Flora of Somalia.

Aloes are popular as house and garden plants in both tropical and temperate climates. The curative nature of most aloes has also been exploited in both traditional and modern societies. Use of aloe products has been documented in early Egyptian civilizations (Dagne, 1996). The sap of *Aloe lateritia* is used in some communities in Kenya for treatment of eye ailments. In modern times, aloes are important in the horticultural industry (Carter, 1994). In South Africa, a multimillion rand industry thrives on exploitation of *Aloe ferox* for commercial uses. Dried leaf exudate of this plant is also used in many traditional medicines and the surplus exported (Van der Bank *et al.* 1995)

At maturity, the variation in size and morphology of aloes is enormous. Some like *A. myriacantha* (grass aloes) are very small in size while others like *Aloe ballyi* grow into tall trees. As noted by Smith *et al.* (1995), both generic delimitation and species concepts in the Aloaceae have been a subject of much discussion. Inter-gradation among populations together with extensive hybridization further complicates the assessment of character variation for taxonomic purposes (Viljoen & van Wyk, 1998). These factors are accentuated by the poor state of most herbarium specimens of aloes, which more often than not lack the accompanying field notes (Sebsebe & Gilbert, 1997).

Reynolds' two books (Reynolds, 1950 and Reynolds, 1966) constitute a monograph that is an important benchmark for the study of aloes. In his second book he provided detailed accounts of aloes then known outside South Africa. Most of the accounts were based on material from the type localities. Reynold's classification exploited the fact that aloes form a natural assemblage with many unifying characters (Smith & van Wyk, 1992). It is on the basis of this that aloes were grouped into 20 groups, based on common character (s) or character combinations (Table 1). However, it should be noted that Reynolds did not study the genus *Lomatophyllum*, whose species are now included in *Aloe* (Rowley, 1996).

Table 1 *Aloe* groups according to Reynolds (1966)

Group/section	Section/ series	Description	Type species
1. Grass aloes	*Graminialoe*	Small acaulescent plants	*Aloe myriacantha*
2	*Leptaloe*		*A. nutii*
3	*Bulbiformes*	Plants with underground bulbs	*A. buettneri*
4		Plants with stripped perianth	*A. peckii*
5		Plants small with compact rosettes	*A. dorotheae*
6	*Series saponariae*	Perianth with pronounced basal inflation, abruptly constricted above the ovary, then enlarging to the throat.	*A. lateritia*
7	*Series Hereroensis*		*A.hereroensis*
8	*Aethiopicae*	Perianth trigonously indented above the ovary	*A. chabaudii*
9	*Verae*		*A. barbadensis*
10		Plants pendent or semi-pendent	*A. veseyi*
11	*Latebracteatae*	Bracts large, broadly ovate or suorbicular	*A. cryptopoda*
12			*A. christianii*
14	*Ortholophae*	Plants with oblique racemes, flowers± second	*A. secundiflora*
15		Racemes densely flowered, bottle-brush like, flowers sessile or very shortly pedicellate	*A. aculeate*

Table 1 continued

16		Medium to large plants with densely rosulate leaves forming rather compact rosettes	*A. percrassa*
17		Medium to large plants with leaves spreading to recurved, slightly to deeply canaliculate	*A. megalacantha*
18		Plants tall-stemmed, simple or few-branched from base	*A. volkensii*
19		Shrubs	*A dawei*
20		Tall trees, dichotomously-branched and rebranched	*A. eminens*

Group 6 of Reynold's aloes is composed of the series *Saponariae*, an assemblage mainly identified on the basis of leaf and floral characters. Plants in this group have thin and flexible 'skin' and are soft to the touch (Gilbert & Sebsebe, 1997). Some workers like Walker (1991) refer to them as 'maculates', a reflection of the spotted leaves. This group is also unified by the characteristic basal inflation of the perianth, followed by an abrupt constriction above the ovary and the eventual enlargement to the throat (Reynolds, 1966; Walker, 1991).

About 40 species of maculates have been described, thirty-two of which occur in Southern Africa. About ten species of maculates occur in Tropical Africa (Table 2). Four of these also occur in South Africa. Members of this group have been reported to occur at various altitudes between sea level and 2700 m (Reynolds 1966). Some like *Aloe zebrina* Bak are widely distributed while others like *A kilifiensis* are endemic to Kenya (Carter, 1994). No maculates have been recorded on the Island of Madagascar (Reynolds 1966, Walker, 1991).

Table 2: Maculate (Reynold's group 6 aloes) Species found in Tropical Africa

Species	Distribution
Aloe swynnertonii Rendle	Mozambique, Malawi
A. saponaria (Ait.) Haw.	Zimbabawe (Inyanga)
A. duckeri christian	Tanzania, Malawi, zambia
A. graminicola Reynolds	Kenya
A. kilifiensis chrstian	Kenya
A. amudadensis Reynolds	Kenya &Uganda
A. macrocarpa Todaro	Benin, Ethiopia, Eritrea, Somalia, Sudan, Nigeria
A. greatheadii Schonl.	Congo & Mozambique,
A. lateritia Engler	Ethiopia, Kenya & Tanzania
A. zebrine	Mozambique, Malawi, Zambia

Source: Reynolds (1966) with updates from Carter (1994) and Sebsebe & Gilbert (1997)

1.2 Literature Review

In its inception by Linnaeus (1753), the circumscription of the genus *Aloe* L. included species that were later to be separated into the genera *Haworthia, Gasteria* and *Kniphofia*. The present circumscription of the genus was given by Duval (cited in Reynolds, 1950) and later upheld and updated by Baker (1881). Later workers also recognized the group under various classifications. Engler (1908) (cited in Reynolds 1950) recognized it as section *Aloineae* in Asphodelaceae.

In spite of its many shortcomings, Reynolds' (1966) account remains the most complete piece of work on the genus *Aloe* in tropical Africa and Madagascar. Using morphological criteria the author identified 20 groups, some natural while others are heterogeneous assemblages without clear demarcation of species boundaries (Table 1). In fact some authours have termed this work as being outdated and therefore in dire need of revision (Walker, 1991).

Since his earliest works, Reynolds (1966) noted the inconsistency in morphological features used in the identification of aloes. Carter (1994) expressed the difficulties encountered in any attempt to put aloes in phylogenetic sequence. Gilbert and Sebsebe (1997) referred to some species groups as being vague with some species misplaced. The identification process is further complicated by extensive hybridization within the genus, which masks the patterns prior to hybridization (Smith *et al.*, 1995; Van der Bank & Van Wyk 1996).

In view of the above classificatory difficulties, Smith and Van Wyk (1992) stressed the need to combine various approaches with morphological ones in order to solve generic and species problems within the family Aloaceae. The imbalance in *Aloe* research is notable with highest concentration in South Africa and fewer studies in Eastern and West Africa. Besides, only a few groups have been the subjects of the studies. For instance, no detailed studies have been carried out on Reynolds' group 6-the maculates.

1.2.1 Karyological Studies

Chromosome number and morphology are recognized as an important tool in plant systematics. As early as 1945, Müller (cited Reynolds 1950) noted that apart from *A. ciliaris*, which is hexaploid, most other aloes are diploids. Newton (1970) in his survey of four West African species confirmed the uniformity in the karyotype, with a basic number n=7.

Cutler *et al.* (1980) in their multidisciplinary study of 12 aloes from Congo, Kenya, and Tanzania also confirmed the constancy in karyotype (x=7). They however recorded tetraploidy (2n=28) in some of the shrubby species and aneuploidy (reduction or increase in the number of chromosomes due to deletion or duplication) (2n=27, 2n=29) in a number of them. They observed autopolyploidy in hybrids of *Aloe kedongensis* and *Aloe dawei*. Based on these findings, they deduced that the tetraploid group is of common origin and that doubling of chromosomes of the ancestral diploid occurred only once. The present situation was assumed to have occurred as a result of diversification.

Hybridization in the genus *Aloe* is a common phenomenon. Smith and Van Wyk (1998) attribute the extensive hybridization in species of *Aloe* to the uniform and bimodal nature of the karyotype. In his analysis, Brandham (1971) showed that there is increased gradation from smaller chromosomes in species in which a number of plesiomorphic (primitive) characters have been retained to larger ones in species with morphological apormorphis (advancement).

Generally, identification of relationships in the genus *Aloe* based on cytology is difficult and may only be practicable by observing the meiotic behaviour among hybrids. As shown by findings of these workers, karyology can be used in classifying the shrubby species of *Aloe* with supporting evidences from other disciplines (Cutler *et al.* 1980).

1.3 Micromorphology

The invention of the light microscope was a tremendous step forward in scientific research in general and botanical studies in particular. For about a century, taxonomists have used the compound light microscope to view minute floral structures. The invention of the Scanning Electron Microscope (SEM) brought even more advancement to the understanding of plants in that it bridged the gap between external micromorphology and anatomy. Using the SEM, botanists have observed with clarity features of the epidermis, seed coats and trichomes (Stuessy, 1994).

1.3.1 Leaf Cuticular Relief

Newton (1972) studied the relief patterns of the leaves of *Aloe macrocarpa* Tod. var. *major*, *A. schweinfurthii*, *A buettneri* and *A. keayi*. The results of this study indicated that relief patterns in the taxa were species-specific. From this work it was concluded that in cases where sufficient populations were available, cuticular relief features are useful taxonomic characters. His results were in agreement with those of Cutler (1969) who had earlier observed and recommended the use of cuticular features in taxonomic diagnoses.

In a multidisciplinary research by Cutler *et al.* (1980), it was found that the cuticular pattern on the epidermal cells of mature leaf surface is uniform within species and is under precise genetic control. They also stated that the pattern is similar in most species, invoking evolutionary or ecotypic linkage.

1.3.2 Anatomical Studies

Working at the Jodrell Anatomical laboratories, Cutler (1972) found that the histology of aloe leaves is relatively uniform. However, it was also noted that characters like the caps to vascular bundles, the cutinized walls, the stomata and epidermal patterns could prove to be useful in diagnosis or even of taxonomic importance. As found in most other members of the family Aloaceae, the cells next to the stoma pore have been shown to be clearly distinct from those not in contact with it in that they have distinct lobes. The stomata are morphologically tetracyclic (Dahlgren & Clifford, 1982). The epidermal patterns were found to be diagnostic with samples (populations) of the same species exhibiting similar patterning.

Beaumont *et al.* (1986) investigated the relationship between the anatomy of the leaf and the type and volume of leaf exudates. It was found that three types of cells could be found at the phloem pole of the vascular bundles. These were identified as aloin cells, outer bundle sheath cells and fibres. Species with fibres produce only sparse exudate, while the majority of species contain aloin cells, which were thought to be storage in function

Smith & van Wyk (1998) noted the presence of thin-walled parenchymatous cells in the inner bundle sheaths in many genera of Aloaceae. They however observed that these characters show general trends in the family but were of no diagnostic value at generic level. In their investigation of the floral anatomy of *Aloe*, *Haworthia* and *Gasteria*, they also observed that the tepals of the perianth are 3-traced, suggesting a tendency in the alooidae towards the development of an inferior ovary, based on amongst others, adnation of the outer floral whorls with the ovary especially in the genus *Haworthia*.

1.3.3 Pollen Morphology

The number, shape of pollen apertures and wall ornamentation are important characters in classification. These studies have been made possible through the invention of the compound Light microscope (LM), the Scanning Electron Microscope (SEM) and the Transmission Electron Microscope (TEM) (Stuessy, 1994).

In the family Aloaceae, most genera have been studied well palynologically (Smith & Van Wyk, 1998). It is reported that sulcate (furrowed) pollen is universal in the genus, while the tectum varies from being sparsely perforate (with pitted surface) in some genera to densely perforate in others. The ektexine consists of a foot layer, intertectal columella and tectum. Some genera are reported to have abrupt transition from perforated sexine to a smooth surface with no perforations in the apertural region.

In search of taxonomic characters in the Aloaceae, Steyn *et al.* (1998) realized that pollen morphology was as yet unexploited in classification. Based on Erdtmans (1969) observations that aloe pollen is monosulacate (single-furrowed) and of medium size, they investigated this character state in 36 *Aloe* species as a pilot survey. Their results showed that pollen grains of *Aloe* species were shed as monads, are of medium size, more or less elliptical in shape, heteropolar and monosulcate with a distal colpus and a perforate to microreticulate tectum.

Muri were smooth. Based on these parameters, species were categorized as belonging to three pollen types, *Aloe albida* type, *A ciliaris*-type and *A. dinteri*-type. These compared well with some categories of Reynolds' (1950) classification of the genus. They therefore recommended more comprehensive SEM studies to evaluate their findings and that pollen data should be used in combination with other sources for species diagnosis.

1.4 Chemical Studies (Phytochemistry)

Chemotaxonomy is a relatively new field with its beginnings in the 1960s and is founded on the principle that similar plants contain similar (chemical) properties (Stace, 1989; Stuessy, 1994). Three types of compounds are of possible occurrence in plants. These are primary metabolites, secondary metabolites and semantides. Primary metabolites are those that take part in essential pathways and are of universal occurrence. They are not useful as taxonomic characters. Secondary metabolites perform non-vital functions and are of limited distribution while semantides may be primary (DNA), secondary (RNA) or tertiary (Proteins). Thus the presence or absence of such metabolic compounds may be useful in detecting relationships among groups of aloes.

The phytochemistry of aloes has interested many workers for several decades. It has been reported that since the mid nineteen-sixties, *Aloe* and other genera have been the subject of many phytochemical studies. Members of the genus contain various compounds in their leaves, including anthronones, chromones, phenylpyrones, phenolic amines and alkaloids. The roots of aloes are reported to contain 1-methyl-8 hydroxyantraquinones (Smith & van Wyk, 1998). According to Dagne (1996), anthrones are the most important class of compounds found in aloes. The tree most important constituents of commercial aloe are the anthrones aloin A and B and the chromones aloesin and aloeresin A.

The analysis of plant chemistry has been made possible through the development of powerful analytical tools (Sivaranjan, 1991). These include High Pressure Liquid Chromatography (HPLC), Thin Layer Chromatography (TLC), protein electrophoresis and DNA techniques. Cutler *et al.* 1980 in their multidisciplinary study of 12 aloes from Congo, Kenya, Uganda, and Tanzania analysed leaf exudates by the use of thin layer chromatography. Based on their results, nine exudate patterns were identified, which correlated with results from anatomy, cytology and the geographical distribution of taxa.

Viljoen *et al.* (1996) combined morphological, HPLC and horizontal starch-gel electrophoresis to unravel the relationships between *Aloe candelabrum* and A. *ferox*. Results from HPLC showed that in its geographical variation *A. ferox* showed very little variation in the composition of major chromone and anthrone derivatives. The main components of *A. ferox* were found to be aloesin C, 5-hydroxyaloin A and two isomers each of aloin and its rhamnosides.

As part of a general chemotaxonomic survey of 380 taxa of aloes, Viljoen *et al.* (1999) demonstrated the presence of plicataloside (a naphthaline derivative) in 20 of the taxa, 17 of which were restricted to East Africa and only 3 to South Africa. This compound was absent in species that are endemic to Madagascar. Some of the species associations suggested by this chemotaxonomic evidence conformed to morphological similarities while many of the taxa had not been previously associated together. From these and previous observations, chemotaxonomy has been shown to as being useful in providing evidence for taxonomic placements within the genus *Aloe*.

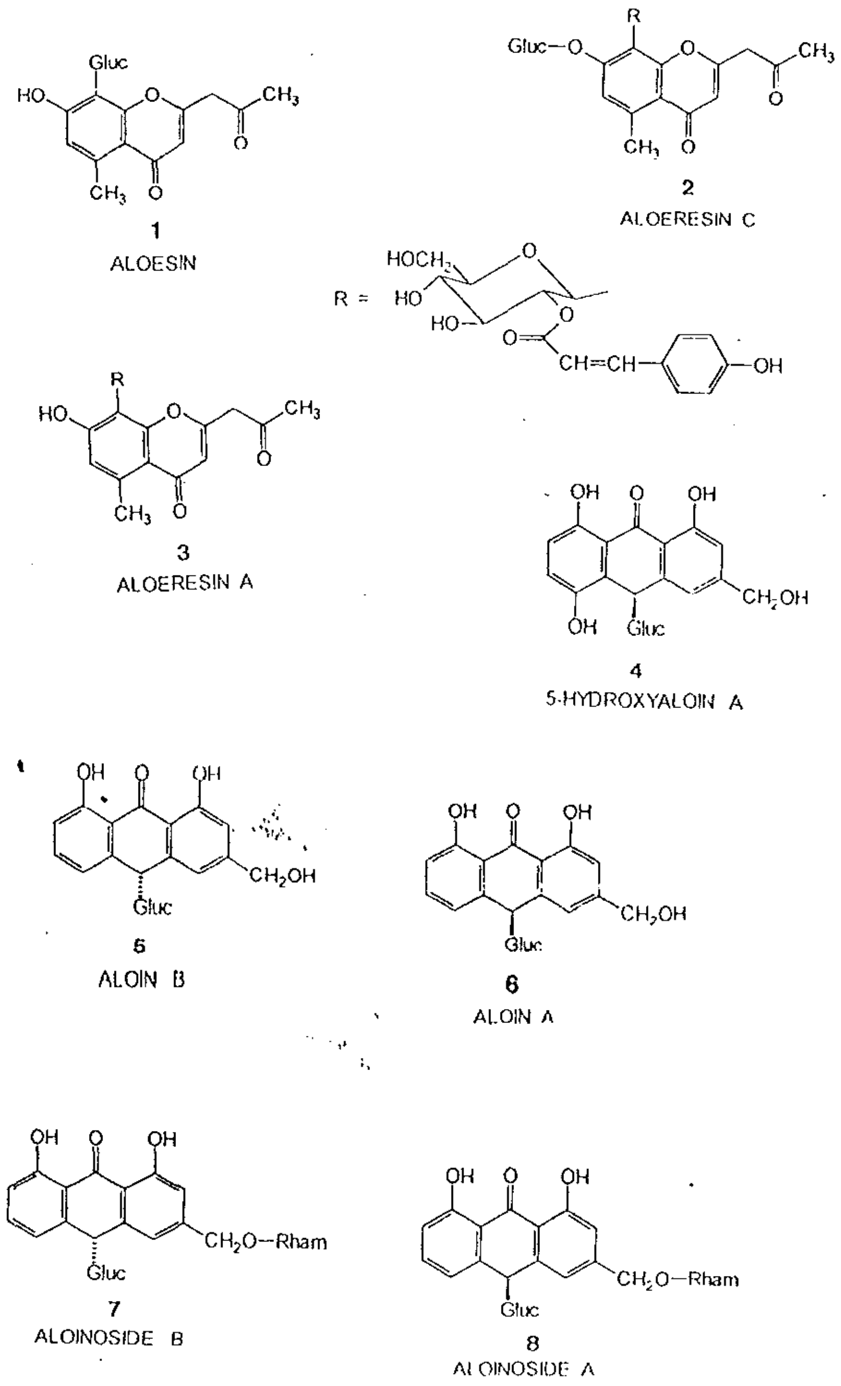


Figure 1: Some Compounds Found in the genus Aloe
Source: Viljoen *et al.*, 1996

1.5 Population structure and Dynamics

The population is recognized as the basic unit of evolution. For this reason, an understanding of the various processes that take place within and between populations is essential to biosystematics research. According to Hutchings (1997), plant population structure results from interactive forces of both biotic and abiotic factors that shape individuals or may have acted on the ancestors. Such forces are also known to affect the genetic structure of populations and mould their performance. The age structure of any population reflects both past opportunities for recruitment and the mortality risks that each recruit has subsequently been exposed.

Population dynamics is concerned with how biotic and abiotic factors interact to bring about changes in plant numbers through time and space. Through quantification of mortality and fecundity, emigration and immigration (in animals, population biologists have been able to explain rarity and abundance in populations as well as the underlying genetic mechanisms.

As noted by Watkinson (1997) and Begon *et al.* (1996) plants exhibit modular growth in which case neither timing nor form is predictable. Modularity in plants is evident at various levels. In some case, individual modules are capable of separate existence and are referred to as ramets. It is thus evident that births in a population may be due to addition of new ramets or new genets (products of zygotes). Begon *et al.* (1996) emphasize the fact that in population studies, the distribution and abundance of ramets may be more important than following the fate of genets.

Evolutionary biologists reckon that the species is a part of a continuum of genetic distinctness. They also emphasize the fact that the evolutionary potential and resilience of species depend on the amount and structure of genetic variation within populations. For this reason, contemporary conservation strategies have shifted emphasis from numbers of organisms to maintenance of genetic variability of species.

In trying to investigate the nature of variation within and between species, examination of ecological characteristics as well a plant-environment interactions gives an insight into the evolution and diversification (Sivaranjan, 1991). Most taxa exhibit predictable patterns of distribution, which is one aspect of their taxonomy. Some taxa occupy mutually exclusive areas and are termed allopatric while those occupying similar or overlapping areas are termed as being sympatric or partially sympatric. The ecological, genetic and structural differentiation exhibited by closely related sympatric taxa is different from that expressed by closely related allopatric taxa (Stace 1989).

Many species exhibit wide phenotypic variation as a result of the plastic responses of individuals to factors of the environment. The vulnerability of plants to environmental pressures such as temperature, water, light and nutrition is accentuated by their stationary habit. This plasticity in plant responses has also been shown to vary considerably, with features like leaf arrangement and floral structures being constant (Davis & Heywood, 1963). Snaydon (1973) noted that a significant amount of diversity in plant species is maintained without the intervention of isolating mechanisms, and sometimes the differences between populations in response to environmental variables are as big as those between ecologically diverse species.

There have been observed differences in phenotype that arise from light intensity that modify form, habit and even the anatomy of individuals. For instance, features of stem elongation and internode length, branching, leaf shape and thickness and flower colour all vary according to light intensities. Shaded plants usually have longer internodes, little branching and more elongated leaves. All the other environmental variables like wind, soil, and temperature together with biotic influences result into phenotypic variations.

In order to investigate the genetic basis of variations, earlier workers like Clements (1922) ventured into the fields of experimental taxonomy. Clements carried out transplant experiments of individuals from the same populations into different ecological conditions. Others like Turreson (1922) grew plants from different areas with different ecological conditions in a controlled garden (cited in Sivaranjan, 1991). These and other works after them led to development of different perspectives of species and speciation (Sivaranjan, 1991).

Ratsirarson (1995) investigated the pollination biology and pollination ecology of *Aloe divaricata* (an endemic of Madagascar). The flowers were found to be hermaphrodite and protandrous and were primarily visited by souimanga sunbirds (*Nectarinia souimanga*) and stingless bees (*Trigona sp.*), the former being most effective in pollination.

Stokes & Yeaton (1995) in their pollination experiments on populations of *Aloe candelabrum* showed that birds are the only effective pollinator guild in the species. They also found that the reproductive fitness of *A. candelabrum* is dependent on the number of plants flowering per unit area and there is selection for limited seed dispersal. *A. candelabrum* was found to exhibit clumping in young populations, with clumps acting as nuclei from which plants spread slowly over time with mature individuals forming centers of the densest stands. Limited seed dispersal resulted in intense competition and consequently self-thinning within the stands.

Midgley *et al.* (1997) studied the population structures of *Pachpodium namaquanum* (Apoocynaceae), *Aloe dichotoma* and *A. pillansii* in Richtersveld, Springbok and Nieuowoudtville in South Africa. Their results showed that both *Aloe* species had high proportions of dead individuals in the Ritchtersveld and all three species had only few seedlings there. Mortality here was attributed to destruction by baboons. The populations in the other localities had low levels of mortality.

1.5.1 Isozyme electrophoresis

The respective discoveries by Harris (1966) (cited in Hubby & Lewontin) in humans and by Hubby & Lewontin (1966) in *Drosophila* of genetic polymorphism for enzymes within the same population triggered off an interest in the use of isoenzymes in biochemistry research. Isoenzymes are defined as variant forms of an enzyme having different primary structures but catalyzing the same reaction. Although different methods are used to detect presence of enzymes, electrophoresis is the method of choice for most genetic analyses (Pollock *et al.* 1987).

The study of isoenymes is thought to bear directly on isolating mechanisms and the reproductive behaviour of taxa. Plant taxonomists, geneticists and evolutionary biologists recognize it as being a reliable and objective criterion for assessing differences between individuals and populations since it makes possible the estimation of the products of gene action rather than their final morphological expression (Kephart, 1990.).

In taxonomical studies, enzyme electrophoresis is useful in revealing relationships between problematic taxa. As noted by Stebbins (1989), this technique can be employed to investigate whether two sets of populations that are sympatric and closely similar in morphological characteristics do or do not share the same gene pool. Positive results have been reported in taxa such as *Chenopodium* (Family Chenopodiaceae). It can also be used in ranking of entities as different subspecies or distinct species, which reflects actual genetic relationship. They also are useful in providing information that relates to hybridization and duplication, including polyploidy. However, it is not yet clear how much genetic differentiation is necessary for species to be regarded as distinct genetic systems.

Genetic analysis distinguishes between two types of variation. The first kind of variation is due to variants coded at different loci within the genome and may have different locations within the cell or may be synthesized at different times during development. These are of universal occurrence in all populations. The second and most frequently used type of variation is due to the presence of different forms of a polypeptide coded at a single locus and often differing in only a few amino acid residues (allozymic variation). The expression of allozymes in an individual as a multi-banding pattern on an electrophenogram depends on the number of alleles and heterozygosity at the locus involved, the ploidy level and the number of polypeptide chains in the functionally active enzyme.

Both ecologists and evolutionary biologists recognize the patchy structure of plant populations. This is attributed to environmental heterogeneity, a factor that leads to clustering of plants within suitable microhabitats. Similarly, it has been demonstrated that like the plants, their genes and genotype tend to be non-random, thus amounting to the genetic structure of populations (Pollock *et al.*, 1987).

In the use of isoenzymes to estimate variation levels, the most commonly used measures of intrapopulational variation are percent polymorphic loci, the number of alleles per locus, effective number of alleles per locus and the mean proportion of loci heterozygous per individual, which refers to the mean heterozygosity assuming Hardy-Weinberg equilibrium. Other statistics often used include the number of alleles per polymorphic locus, the observed proportion of loci heterozygous per individual and measures of genetic diversity equivalent to the Shannon species diversity index (Hamrick, 1989). For instance Nei's (1972 & 1978) diversity indices are used in measuring genetic distance and similarity between populations and species.

The average number of alleles is a score of all alleles at a locus irrespective of their frequencies. For percent polymorphic loci, an arbitrary level like 95% is used to determine as to whether the locus is polymorphic (A locus is considered polymorphic if the frequency of the most common allele is less than 95% or 99%). Both of these measures are unreliable since they are influenced by sample size. Average heterozygosity is a measure of the proportion of the population that is heterozygous.

It has been reported that plant populations generally maintain relatively high levels of allozyme variation within populations. A review by Hamrick & Godt (1989) of over 100 species revealed that an average plant species has 37% of its loci polymorphic, 1.69 alleles per locus and mean heterozygosity per individual of 0.141. Within-population variation was also noted to be heterogenous. This was associated with the life history and ecological characteristics of the species. Higher levels of intrapopulation variation were associated with species that were primarily out-crossed by wind pollination. Table 3a & 3b below show the amount of variation at population and species levels according to Hamrick & Godt (1989) with values of the standard error in parenthesis.

Table 3a Variation at Species level

	All plants (general)	Monocots	Endemic taxa	Tropical taxa	Out-crossing/ Animal pollinated	Long-lived perennial herbs
P	50.5 (1.4)	40.3 (3.0)	26.3 (2.1)	32.7 (3.0)	35.9 (1.8)	39.3 (16.2)
A	1.96 (0.05)	1.66(0.08)	1.39 (0.03)	1.45(0.05)	1.54 (0.03)	1.44 (0.20)
A_e	1.21 (0.01)	1.21(0.03)	1.09(0.01)	1.13(0.02)	1.17 (0.07)	1.14 (0.05)
H_e	0.149 (0.006)	0.144(0.012)	0.063 (0.063)	0.109 (0.012)	0.124 (0.008)	0.084 (0.028)

P = % polymorphic loci A= no. of alleles A_e= effective no. of alleles H_e = Expected heterozygosity

Table 3b Variation at Population Level

	All plants	Monocots	Endemic taxa	Tropical taxa	Out-crossing/ Animal pollinated	Long-lived perennial herbs
P	34.2(1.2)	59.2 (3.4)	40.0 (3.2)	49.2 (3.6)	50.1 (2.0)	39.6 (16.5)
A	1.53(0.02)	2.38(0.17)	1.80(0.08)	1.81 (0.10)	1.99 (0.07)	1.42 (0.13)
A_e	1.15(0.01)	1.27(0.03)	1.15(0.04)	1.21 (0.03)	1.24 (0.02)	1.28 (0.12)
H_e	0.113(0.005)	0.181 (0.0015)	0.096(0.10)	0.148(0.015)	0.167 (0.10)	0.205(0.084)

P = % polymorphic loci A= no. of alleles A_e= effective no. of alleles H_e = Expected heterozygosity

The use of protein electrophoresis in taxonomical studies in the family Aloaceae is a relatively new endeavor. Campbell *et al.* (1995) undertook a feasibility study on the use of starch gel electrophoresis in determination of the amount of genetic variation within and between *Haworthia pumila* and *H. herbaceae*. The results of the study indicated low levels of genetic variation. This was in agreement with those of Van der Bank *et al.* (1995) in which very low levels of genetic variations were recorded in populations of *Aloe ferox* and *A. marlothii* respectively (0% & 4.7% polymorphic loci). These observations led to recommendations of more extensive electrophoretic survey in the family Aloaceae.

In a bid to establish the progress of hybridization and phylogenetic relationships in the genus *Aloe*, Van der Bank & Van Wyk (1996) carried out an electrophoretic survey on populations of *A ferox*, *A. arborescens* and their hybrids. Their investigation showed that hybrids exhibited character coherence by sharing the species-specific alleles of pure species. In the same study, genetic markers for identification of hybrids were identified.

Viljoen *et al.* (1996) investigated the taxonomic position of *A. candelabrum* and *A. ferox* by isoenzyme analysis. The study material was extracted from geographically isolated populations of both taxa. Using Nei's (1972) measure to estimate genetic similarity and distances between the populations, the two taxa were found to be conspecific.

1.6 Statement of the Problem

The *Aloe saponaria* group of plants can be distinguished from the rest of the aloes based on their characteristic soft 'skin' and maculation together with the basal swelling and abrupt constriction of the perianth. However, as observed by earlier workers, delimitation of species boundaries is often problematic (Reynolds 1966; Walker, 1991)

Reynolds (1966) observed that the group is highly heterogeneous, making construction of a watertight key for all species an unforeseeable possibility. There is variation in size of plants and the extent of maculation depending on altitude, soil, rainfall and season. For this reason, the maculates represent a taxonomic complex with inter-twined morphologies. This study was an attempt to unravel two members of this complex-*Aloe macrocarpa* Tod. and A. *lateritia* Engl.

1.6.1 Taxonomic position

Reynolds (1966) treated *Aloe macrocarpa* as having two varieties i.e. *A. macrocarpa* var. *macrocarpa* and *A. macrocarpa* var. *major*. The typical form of *A. macrocarpa* var. *macrocarpa* (from Tigray region, Ethiopia) was described as having leaves 20-30cm long and 6-7cm broad, growing singly or in groups. Racemes ranged in length from 15-20 cm with perianth averaging 25mm. The latter variety was distinguished from its counterpart in having larger flowers (20-30 cm long) and racemes a little longer and lax.

In Reynolds' account, *Aloe graminicola* Reynolds and *A. lateritia* were treated as separate species. In his commentary however, Reynolds stated that *A. gramininicola* is closely allied to *A. lateritia*, but differed from it in having smaller, denser racemes of much narrower, more curved flowers with the basal swelling of the perianth being much narrower. He also observed that the type of *A. lateritia* was an outlier in having short, almost capitate racemes since the type population had plants that exhibited wide variation in leaf size and spotting, length of racemes and of flower. Deviations from the typical "coral red" colour of flowers were also common.

Carter (1994) treated *A. graminicola* and *A. lateritia* as being variable forms of the same species. Thus she recognized *A. lateritia* var. *lateritia* and *A. lateritia* var. *graminicola*, the separation being mainly due to the shape of the raceme, which tends to be more capitate in *A. lateritia* var. *graminicola*.

Sebsebe & Gilbert (1997) recognize *A. macrocarpa* at specific level without any variety. In their account they indicated that the basis of separation of *A. macroacrpa* from *A. lateritia* in the treatment for the Ethiopian Flora is ambiguous since the nature of morphological variation between members (populations) of the two species is continuous.

From the above accounts it is evident that on the basis of morphological criteria, there is dissenting opinion as to the validity of the separation of the two taxa. The review of earlier works is emphatic of the fact that taxonomic relationships in the genus *Aloe* generally and in the *A. saponariae* group specifically are complex and no single evidence can suffice for disentangling the species complexes. This research was an attempt therefore, to unravel one of the taxonomic complexes by using a multidisciplinary approach. Detailed descriptions of the species are given in appendix 3

1.7 Research Hypothesis

Aloe macrocarpa Tod. and *A. lateritia* Engl. may be conspecific.

1.8 objectives of the study

1.8.1 General Objectives

This study was an attempt to collect data that may justify the merger or the clear separation of the two species and to further investigate the interspecific and intraspecific relationships of *A. macrocarpa* Todaro and *A. lateritia* Engler

1.8.2. Specific objectives

This research aimed to: -

- Review morphological characters previously used in delimitation of taxa
- Study the patterns of variation and population structure of the two taxa.

2.0 MATERIALS AND METHODS

The methodology used in this study falls into several components as described below: -

2.1 Macromorphology

Specimens of *Aloe macrocarpa* and *A. lateritia* were obtained from the National Herbarium of Ethiopia (ETH), the East African Herbarium (EA) and the Herbarium at the Royal Botanic gardens, Kew (K) respectively. Specimens of *A. saponaria* were obtained from the National Herbarium of Zimbabwe (SRGH) for comparison.

Using a hand ruler, measurements were made of both reproductive and vegetative parts (Table 4). Dry floral parts were boiled or soaked in ethanol to soften before being examined. Where necessary, a binocular microscope was used.

Examination of freshly collected specimens was done to augment studies of herbarium specimens. The general habit and the vegetative and floral parts of the plants were studied using the same guidelines as those used for herbarium studies.

Arithmetic means and standard deviations from the mean were calculated for the total number of specimens examined in every taxon (n). The altitudinal ranges of the taxa and variations in morphological characters were presented in the form of histograms.

Table 4: List of morphological features examined per specimen

Collector and Number:
Locality: Altitude and position:
Character
HABIT
Stems +/-
Length
LEAVES
Shape
Length x width
Colour, including maculation
Marginal teeth: colour
No. Per 5 cm (towards base)
Size
INFLORESCENCE
Number and type of inflorescence
Number of branches per inflorescence
Lowest (oldest) peduncle length
Pedicel length in flower
Raceme: Shape
Diameter &Length of raceme
Floral Density of raceme
Sterile bracts-shape and size
Colour and no of nerves
Floral Bracts-shape and size
Colour and no. of nerves
Perianth:
Diameter at base, constriction and above
Colour and full length of lobes
Length fused (outer)
Length free (outer)
Stamens: Filament Colour & length
Anther size & colour
Pistil: Ovary length &width
Style length
Fruit: Length x width
Seed: Colour and texture
Seed diameter
Wing diameter

2.2 Pollen studies

2.2.1 Light microscopy

Acetolysis

Flower buds were collected from both herbarium specimens and living material. These were then crashed in a mortar after addition of a few drops of distilled water. With the use of a 200μ sieve, the resultant suspension was sieved into a 250 ml beaker and then transferred into centrifuge tubes. The contents were centrifuged at 3000 revolutions per minute for 3 minutes, separating the particulate matter from the liquid, which was then decanted off.

- Acetolysis was done using standard guidelines by Erdtman (1969).

Preparation of Reference Slides

To prepare reference slides, glycerin jelly was cut into a number of small cubes. A pinhead-sized cube was taken using a clean needle with the jelly cube and then transferred to a clean, labeled slide. The jelly was melted on a slide warmer, stirring in the process. A cover slip was then placed on the pollen and sealed off with wax.

Observation and photographing of pollen grains was done at x1000 magnification on a laborlux 12 Leitz Microscope fitted with an ocular micrometer (under oil immersion). For each slide, ten pollen grains were examined for shape and size both in polar and equatorial orientation.

2.2.2 Scanning Electron Microscopy (SEM) of pollen grains

The SEM studies of pollen grains were done at the International Centre for Insect Physiology and Ecology (ICIPE)- Kenya.

The grains used for these studies were obtained from dried herbarium specimens and were examined in their natural state without acetolysis (Radford *et al.*, 1974). The list of specimens examined is given (table 5). Anthers were selected and observed under a binocular microscope for signs of pollen dust. A few grains of pollen were then scooped and mounted on metal stubs fitted with double-sided adhesive tape ready for coating with gold and observed under the JEOL-T330A SEM.

Table 5 Specimens used in the SEM study

Collector & Number	Species	Provenance
Brown, E. S. 1751	*A. lateritia* var. *graminicola*	Hyrax Hill- Kenya
Owino, F. 21	*A. lateritia* var. *graminicola*	Ol Joro Orok- Kenya
Reynolds, 6369	*A. lateritia* var. *lateritia*	Rombo- Tanzania
Sebsebe, D. 2945	*A. macrocarpa*	Daleti-Ethiopia
Sebsebe, D. *et al.* 9304	*A. macrocarpa*	Dedeba- Ethiopia

Observation of pollen grains was done at 10KV at magnifications of x2000 and x7500 respectively

2.3 Population Structure and Dynamics (Table 6)

The distributions of the taxa were obtained from herbarium records and the existing literature (Reynolds, 1966; Carter, 1994 & Sebsebe & Gilbert 1997). Based on the position data of the localities, a map was drawn (Figure 8) to show the distribution of the three taxa.

In this study two populations of *A. macrocarpa* and 3 populations of *A. lateritia* were sampled and studied. *Aloe macrocarpa* was sampled at Shashemene and Goha Tsion Shewa, Ethiopia. *Aloe graminicola* var. *graminicola* was studied at Nanyuki and at Gilgil (Kenya). *Aloe lateritia* var. *lateritia* was represented by only one population at Oloitokitok (Kenya) (Table 6). The criterion for selection included accessibility from Addis Ababa and Nairobi respectively with emphasis on populations where morphological variations have been documented.

Table 6 Populations studied

Species	**Population** from	**Altitude (m)**	Latitude	Longitude
A. macrocarpa	Shashemane ((C)	1882	7° 18.15' N	38° 38.7'E'
	Goha Tsion (**D**)	2436	10° 1.77 'N	38°14.65' E
Aloe lateritia* var. *graminicola	Nanyuki (**A**)	2103	0° 3.21'N	37° 2.87' E
	Gilgil (**B**)	2100	0° 32' S	36° 1.9' E
Aloe lateritia* var *lateritia	Oloitokitok (**E**)	1390	0°25'S	37° 35'E

At the onset of the research, plots of appropriate size (5 m x 20 m)^2 were established and mapped out. Each individual clone, consisting of genets and ramets were marked and recorded. Records were made of genet number (G), ramet diameter (RD), rosette height (RH), number of leaves (LN), number and branching of inflorescence. These measurements were made twice during the period of research at six months' interval.

In the second season of data collection, attention was paid to the signs of recruitment within the populations, in terms of both new genets and new ramets. Dead plants were also noted. This data was used in computing and comparing the modes and rates of recruitments amongst the population studied.

Based on the data obtained, the spatial structure of all the populations was presented in the form of histograms of total module number (ramet plus genets) and the frequency of genets (genetic individuals). On the assumption that the number of leaves on a plant correlated directly with its size, the ramets were categorized in 5 leaf intervals. A combined histogram was drawn to compare the size classes in terms of leaf number in the 5 populations (Nordal *et al.*, 1997).

2.3.1 Scanning electron microscope studies on leaf cuticles

These were carried out at the University of Oslo laboratories. It was realized that preparation of *Aloe* leaves according to the usual procedure of fixation and successive dehydration distorted the cuticle. In order to sidestep this misnomer, the abaxial cuticle was carefully peeled off from selected fresh leaves (localities) and mounted on metal stubs. The cuticle was mounted without cleaning or scratching in order to avoid distorting any surface structures like wax, which is known to be present (Cutler, 1969).

These were then coated with gold and examined by use of Jeol-6400 Scanning Electron Microscope. Observation of surface features was done at 10KV and at magnifications that ranged from x100 to x2000

2.3.2 Isoenzyme Analysis

Collection of material

Leaf material from natural populations was used in this analysis. In order to capture maximum variation in the populations, random (cluster) sampling was used. In populations that were large enough, 30 leaves were sampled (only 26 leaves were run for the analysis). Populations at Goha Tsion and at Oloitoktok had about 12 individuals and therefore leaves were collected from all individuals (Table 7).

Table 7 Localities and Sample size of populations used for enzyme electrophoresis

Species	Localities	Sample size (n)
A. macrocarpa	Shashemane(C)	26
	Goha Tsion (D)	12
A. lateritia var. *graminicola*	Nanyuki (A)	26
	Gilgil (B)	26
*A. lateritia.*var. *lateritia*	Oloitokitok (E)	12

Extraction of enzyme

Approximately 1 x 1 cm^2 of leaf area was cut and dissected to remove the lower surface and gel-like substance. The upper surface was then sliced into smaller blocks. These were then put into microfuge tubes to which approximately 7 drops of homogenizing buffer and a few grains of sand were added. The leaf tissue was then ground manually by use of a glass pestle, resulting with a more or less homogenous 'soup'. This was then stored at minus 70^0c. Prior to each electrophoresis, the enzyme 'soup' was absorbed into paper wicks, which were then loaded and run on horizontal starch gels. A summary of the electrophoresis process followed is presented in figure 2.

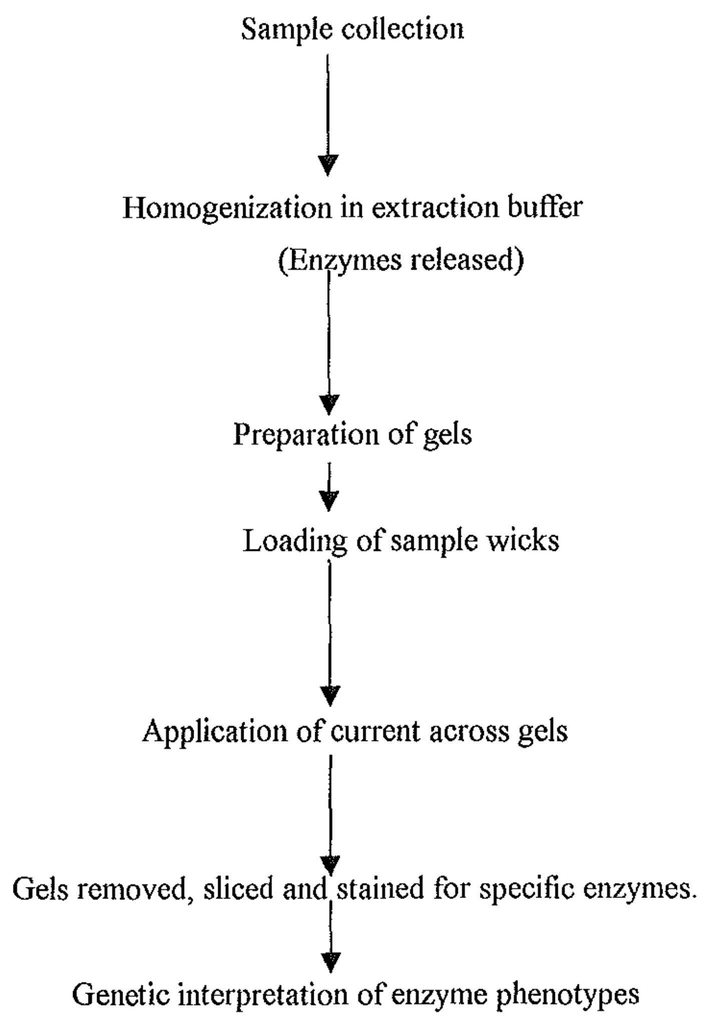


Figure 2 Summary of the steps followed in the Isoenzyme Analysis

The Enzymes assayed for in this study were Aspartate aminotransferase (AAT), Isocitrate dehydrogenase (IDH), Phosphoglucomutase (PGM), Phosphogluconate dehydrogenase (PGD) Malate dehydrogenase (MDH, Triphosphate isomerase (TPI), and catalase (cat) Glucose-6-phosphate (PGI).

Based on band phenotypes of the five populations studied, cluster Analysis was done using SPSS version 9.0 software (figure 16). The collection of tissue, preparation of extraction buffers, electrophoresis, staining of gels, interpretation of results, locus nomenclature follows Soltis and Soltis (1989).

The standard procedures of the POPGENE version 1.31 (A Microsoft Window-based Freeware for Population Data Analysis) were used for population data analysis. The following computations for diploid data were included in the analysis: - Hardy-Weinburg test, fixation index, allele frequency, effective alleles, polymorphic loci, observed homozygosity, observed heterozygosity and expected heterozygosity.

F-statistics (Nei, 1978) were used to partition variance of the allele frequencies into that among versus within populations. Hardy-Weinberg equilibria were tested using the algorithm by Levene (1949).

2.4 Additional data

2.4.1 Ecological and Geological background to the Study Sites

In order to relate the structure and dynamics of the populations studied to the environmental influences under which they grow, it was found necessary to outline the general climatic as well as topographical and endaphic characteristics of the ecological zones in which the populations occur. Information was sought on the general geological structure, rainfall, temperatures and vegetation types of the regions in which populations were sampled (Table 13). Unless otherwise stated, this information was obtained from the National Atlas of Kenya (1970), the Nakuru District Development Plans (1994-1996 & 1997-2000) and the National Atlas of Ethiopia (1988). Rainfall information of Ethiopian Stations was obtained from Gamachu (1977).

Information on individual demographic sites was outlined from personal observation made at the time of the visit (s) to the sites with emphasis on the specific habitat and other species associated with the target taxa. Inquiries were made on the use of the plants by the local community and signs of disturbance were noted.

3.0 RESULTS

3.1 Macromorphology

A sufficient number of specimens were seen for this study (Appendix 1). However, many specimens were incomplete, thus making it impossible to have measurements for every feature as initially planned. It was thus inevitable that morphological data has been presented with many missing values e.g. for bracts, leaves and in some cases style and stamen length. It was generally observed that values for the various measurements displayed extensive overlap in their ranges. These measurements have been summarized and presented in the table below (table 8). Detailed morphological data have been presented in appendix 2.

Table 8: Summary of Quantitative measurements obtained

	A. macrocarpa	*A. laterritia* var. *graminicola*	*A. lateritia* var. *lateritia*
Altitude (m)	1100-2500 (3000)	1500-2499	(70) 250-2590
Leaf Length (cm)	(9) 13-41(53)	8-31.5(41)	20-40(45)
Leaf width (cm)	2.5-7.5	3-7.2	2.7-7.5
No. of marginal teeth	6-16	3(5)-11	5-11
Size of marginal teeth (mm)	1-7.5	1.16-7.5	1-7
Branches per raceme	2-7	2-6	3-7
Peduncle length in flower (cm)	(8)10-25	12-38	15-29(41)
Pedicel length in flower (cm)	1.3-2.7	15-2.76(3.6)	1.2-3.4(4.25)
Raceme length (cm)	(5) 11-22(27)	5-14.5	4-26
Raceme diameter (cm)	4.5-10	5-11.5 (13)	5-10
Length of sterile bracts (mm)	12-35	14-30(50)	16-43
Width of sterile bract (mm)	5-15	3-6 (8)	4-10
No. of nerves per sterile bract	9-11(16)	3-10(13)	6-14
Length of flora bract (mm)	8-17	8-15(30)	8-17
Width of floral bract (mm)	2-12	2-5	2-4
No. of nerves per sterile bract	1-6(15)	2-5(7)	2-4
Perianth diameter at base (mm)	4-9	2-8	4-6
Perianth diameter at constriction (mm)	1.5-2	1-3	1-5
Full length of perianth (cm)	1.8(2.4)-3.5	2.1-3.3	1.6(2.4)-3.9
Length of perianth free (mm)	3-8(13)	5-10	4-9
Stamen length (mm)	(8)16-29	16-28	11(14)-27
Anther length (mm)	3-5	3-6	5
Anther width (mm)	0.5-2	0.5-2	1
Ovary Length (mm)	5-8	5-10	3-10
Ovary width (mm)	1-2	1-3	1-2
Style length (mm)	(13) 19-28	14-26	8(18)-30

Figures in parenthesis represent extreme (rare) values obtained for the taxa

Leaf size

Leaf sizes for *A. macrocarpa, A. lateritia* var *graminicola* and *A. lateritia* var. *lateritia* measured (9) 13-41(53) x 2.7-7.5, 8-31.5(41) x 3-7.2 and 16-40(45) x 2-7.5 respectively. In living material, it was observed that leaves in shaded environments tended to be longer than those in exposed habitats. No consistency was observed in leaf size amongst the populations studied.

Leaf colour and maculation

The colour and maculation of the leaves tends to follow a similar patterns of variation in all populations, with shaded leaves being pale green with fewer spots while exposed plants had various shades of colour between purple and maroon and increased spotting. All the other character states examined showed overlaps (Appendix 2)

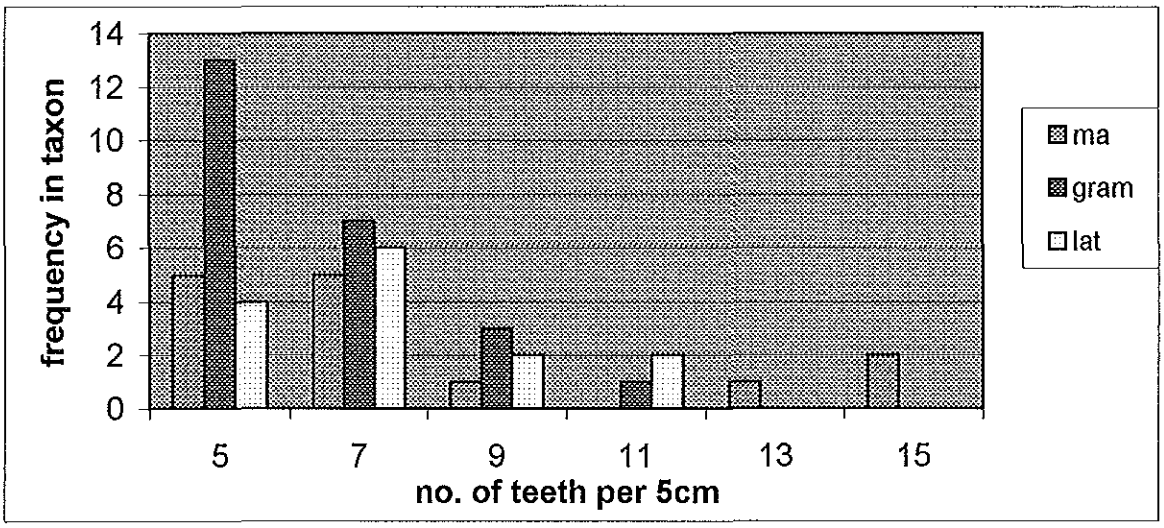
Raceme Shape

Raceme shape in the genus *Aloe* may range from capitate (with dense heads) to subcapitate to cylindrical based on observations of length to width ratio. From observations both in the field and on herbarium specimens, it is true that there is a tendency in some populations towards one kind of raceme shape. However, it was observed that all the three raceme shapes are represented in the taxa. Of the specimens examined, *A. macrocarpa* had capitate: subcapitate: cylindrical racemes in the ratios of 6:7:5 (33%, 38.9% & 27.7%); *A. lateritia* var *graminicola* had 29:4:2 (82.9%, 11.4% & 5.7%) and *A. lateritia* var. *lateritia* 15:7:6: (53.6%, 25% & 21.4%).

Marginal Teeth

The ranges of measurements were 6-16, (3) 5-11 and 5-11 in *A. macroacarpa, A. lateritia* var. *graminicola* and A. *lateritia* var *lateritia* respectively. The mean numbers of teeth per 5 cm was 8.78, 6.96 and 7.68 for *A. macrocarpa, A. lateritia* var. *lateritia* and *A. lateritia* var. *graminicola* respectively (Appendix 4).

The observed variation in specimens of the three taxa is shown in figure 3 below.

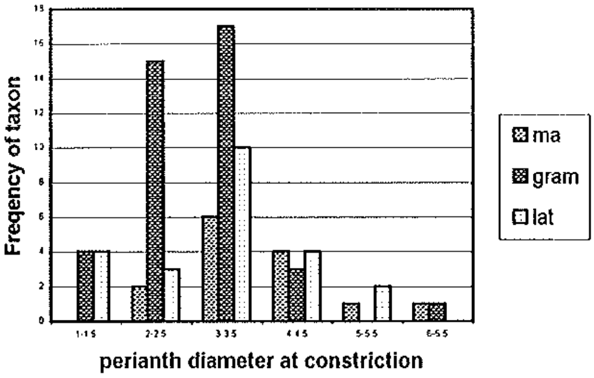


Ma= ***A. macrocarpa gram=A. lateritia*** **var.** ***graminicola*** **lat=** ***A lateritia*** **var.** ***lateritia***

Figure 3: Variation in density of teeth in the three taxa

Perianth diameter

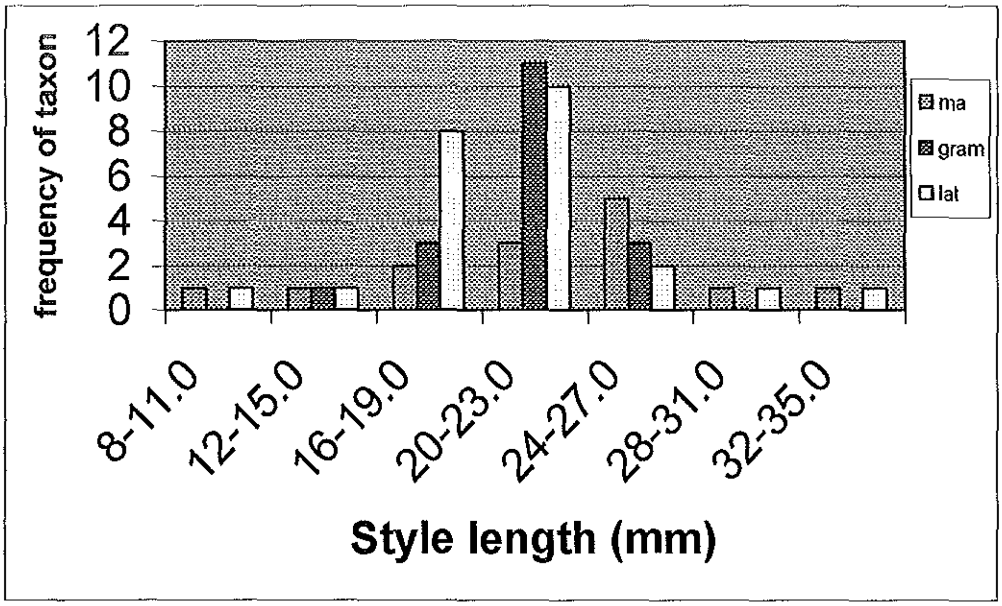
The diameter of the flower was observed at four positions. These were at the base (swollen part), at the constriction, above the constriction and at the open tip of the lobes, with emphasis on the first two levels. In the specimens examined the measurements of ranged from 0.2- 0.8cm (*A. lateritia* var. *graminicola*) to 0.4-0.6 (*A. lateritia* var *lateritia*) to 0.4-0.9cm (A. *macroacrpa*). The overlap was also evident in the size of the perianth diameter at and above the constriction (Fig 4).



ma= *A. macrocarpa* gram= *A. lateritia* var. *graminicola*, lat= *A. lateritia* var. *lateritia*
Figure 4: variation in perianth diameter in specimens of the three taxa

Style Length

Observations of mean style length in specimens of the three taxa were 1.96 cm, 2.2 mm and 2.26 cm in *A. lateritia* var. *graminicola*, *A. lateritia* var *lateritia* and *A. macrocarpa* respectively. The mean style length and deviations from the mean are presented in appendix 5. As illustrated in figure 5, there were no obvious gaps in the measurements of style length in specimens of the three taxa.



Ma= A. *macrocarpa* gram=*A. lateritia* var. graminicola lat=*A. lateritia* var. *lateritia*

Figure 5: Variation in Style Length in the three taxa

3.2 Micro morphological Observations

Pollen characterization was done following guidelines by Erdtman (1969) and Faegri & Iversen, 1975). Pollen grains were described as being prolate when the polar axis is longer than equatorial axis **(P: E= 1.330-2.00)** and perprolate when the equatorial axis is more than half the length of the polar axis **(P: E.2.00).** The measurements obtained are shown (table 9).

Symmetry and Form

All the grains examined were symmetrical in form and monosulcate (with a single furrow). The sulcus was shallow and as long as or approximately equal to the polar length of the grain. The sulcus ends were acute.

Pollen Size

The polar length in *A. macrocarpa* varied from 52.22– 66.08 µm, that of *A. lateritia* var. *graminicola* was 51.2–67.26µm while *A.lateritia* var. *lateritia* measured between 52.2 – 67.226 µm. The equatorial length ranged from 10 (17.7) (*A. macrocarpa*) to 34.78 ųm. The P: E ratio ranged from 1: 1.5 (*A. lateritia* var. *graminicola*) to 1:5.22 (*A..macrocarpa*). All the grains examined were either prolate or perprolate.

Table 9: Measurements and classification of the pollen grains as seen under the light microscope (x400)

Taxon Id./Coll. & no.	Provenance	Shape & class	P.L	EQ.L	P: E
Aloe macrocarpa					
Ensermu *et al.*,252 (ETH)	Shashemane	prolate	58.00	34.40	1:1.68
Sebsebe 2945 (ETH)	Daleti	prolate	58.00	31.909	1: 1.81
Sebsebe *et al.*, 9304 (ETH)	Shashemane	perprolate	66.08	24.78	1:2.66:
Mooney, 9597 (ETH)	Quiha	perprolate	63.72	17.7	1:3.60
Gilbert *et al I*(ETH) 056863	Ded'ede	perprolate	52.2	10.00	1:5.22
Gilbert & Philips 8862 (ETH)	Arba Minch	perprolate	54.28	28.32	1:1.91
Aloe lateritia* var. *graminicola					
Wabuyele &Mbale 40 (ETH)	Gilgil	perprolat	51.92	23.00	1:2.26
Gillett 16268 (EAH)	Nyeri	prolate	60.18	30.68	1:1.96
Kutilek, M, 104 (EAH)	Nakuru	perprolate	54.28	24.78	1:1.45
Perdue & Kibuwa 9270 (EAH)	Nakuru	perprolate	60.18	28.32	1:2.12
Brown, E. S, 1751 (EAH)	Hyrax	prolate	56.64	31.86	1:1.78
Wabuyele & Mbale 17 (ETH)	Nanyuki	perprolate	59.00	21.24	1:2.78
Aloe lateritia* var. *lateritia					
Reynolds 6369 (EAH)	Rombo	perprolate	67.26	25.32	1:2.66
Bally PRO 10311 (EAH)	Ngong	perprolate	52.2	34.8	1:1.5
Reynolds GW, 6977 (EAH)	Voi	prolate	59.00	30.68	1:1.92.

3.2.1 Pollen Ultra-structure

Exine Stratification

All grains examined were tectate with reticulate sculpturing (lumina wider than muri). Slight differences in populations were observed in the shapes and width of lumina and muri (Erdtman, 1969, Moore & Webb, 1978). These observations have been summarized in table 10 below: -

Sulcus

The sulcus was distally located, running the whole length of the pollen grain. It was shallow with acute ends and was bordered by lumina that averaged in size smaller than those further away.

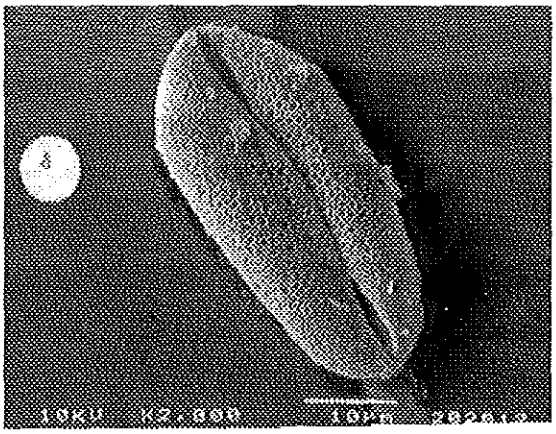
Pollen from Ol Joro Orok (*A. lateritia* var.*graminicola*) had irregularly shaped lumina that averaged 0.92μm with sizes diminishing towards the sulcus (fig.6a-b). Muri size averaged 0.5μm while pollen from Hyrax Hill (*A. lateritia* var. *graminicola*) had lumina and muri that measured 1μm and 0.5μm respectively (fig.6c-d)

Table 10: Measurements of Lumina and Muri in specimens of the three taxa

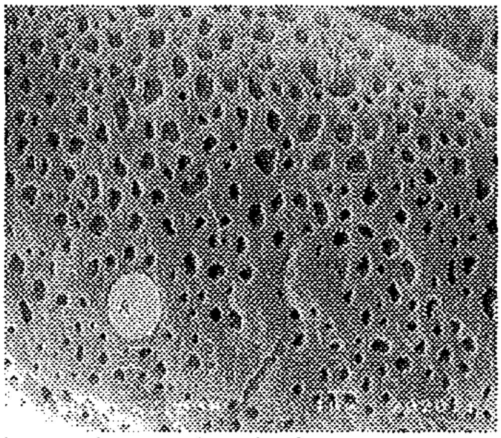
Taxon	Provenance	Lumina shape	Lumina width (μm)	Muri width (μm)	Taxon means (μm) Lumina width	Muri width
A. macrocarpa	Shashemane-Ethiopia	Regular/	0.82	0.42	0.66	0.42
	Daleti-Ethiopia	Regular	0.5	-		
A. lteritia var. *graminicola*	Ol Joro Orok-Kenya	Irregular	0.92	0.5	0.96	0.5
	Hyrax Hill-Kenya	Irregular	1	0.5		
A. lateritia var. *lateritia*	Rombo-Tanzania	Irregular	0.42	0.42	0.42	0.42

Pollen grains from Rombo on the other hand (*A. lateritia* var. *lateritia*) had lumina and muri averaging 0.42μm (Fig. 6i-j). The sulcus ends were less sharp than those observed in pollen from Ol Joro Orok and Nakuru. Lumina in these grains were irregular but generally smaller than those observed in populations of Nanyuki and Gilgil.

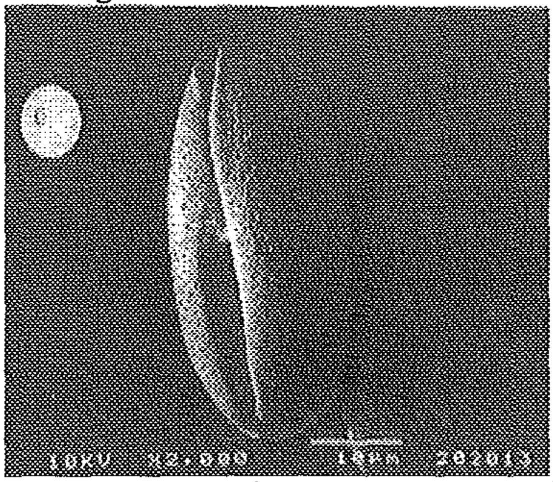
Pollen from Daleti (Fig 6e-f) and Shashemane (Fig 6g-h) (*Aloe macrocarpa*) had lumina that were more often than not regularly rounded. In the pollen grains from Daleti, the diameter of lumina and muri averaged 0.82μm and 0.42μm respectively. Grains from Shashemane had average lumina diameter as 0.5μm.



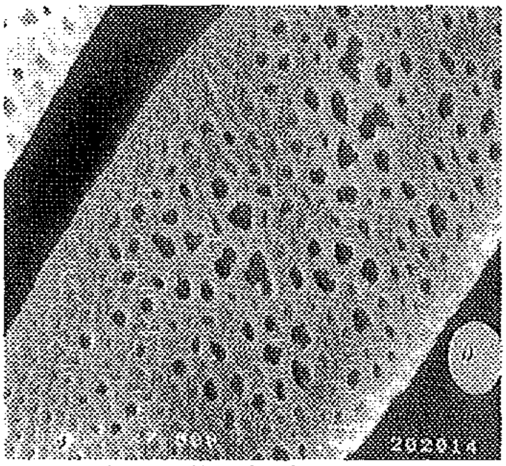
a:Ol Joro Orok, (*A. lateritia* var. *graminicola*) Whole grain



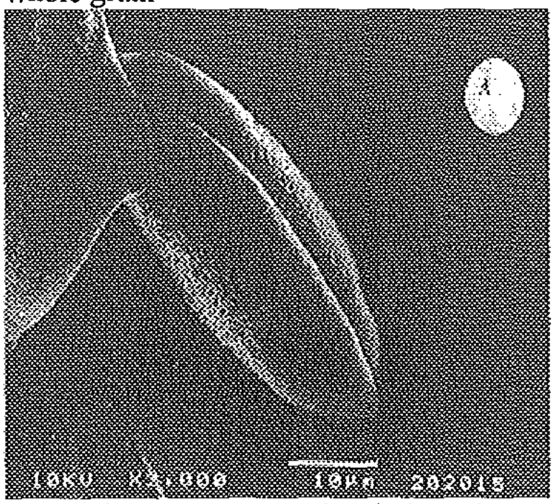
b: Details of exine showing irregular muri



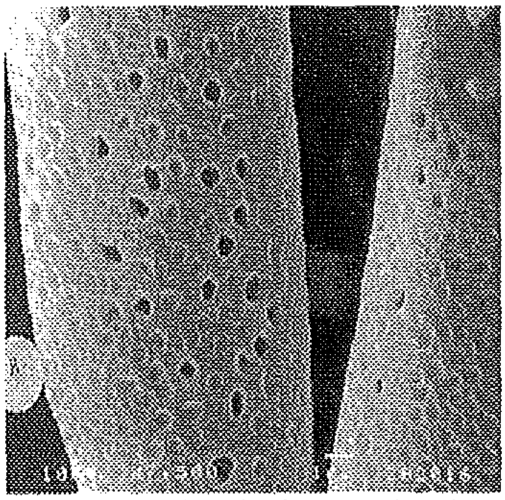
c: From Hyrax (*A. lateritia* var. *graminicola*) whole grain



d: Details of exine: irregular muri

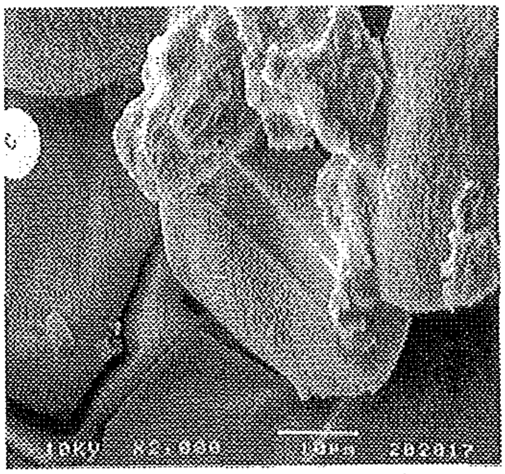


e: Daleti (*A. macrocarpa*), whole grain

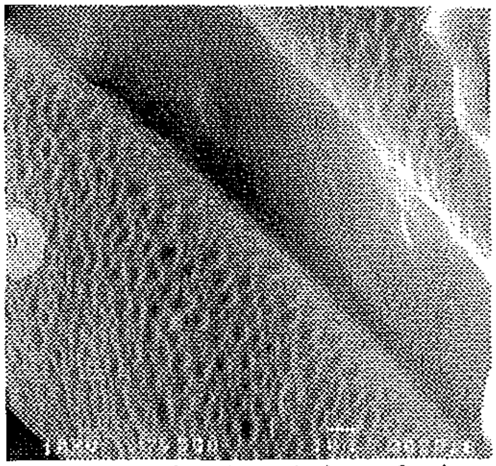


f: Details of exine showing the regular muri

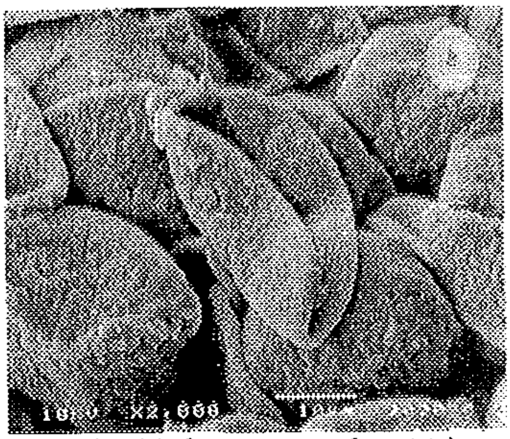
Figure 6: Pollen Ultra-structure



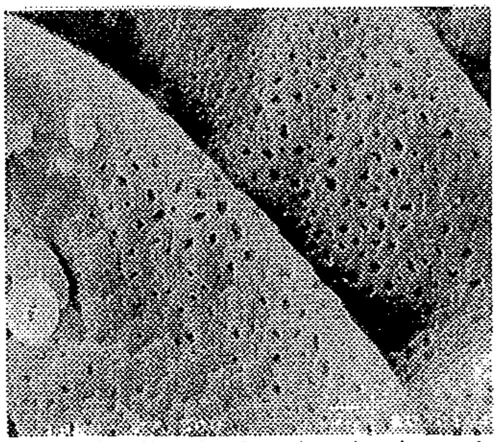
g:Daleti (*A. macrocarpa*), whole grain,



h: Blurred view of exine detailspartially visible



i: Rombo (*A. lateritia* var. *lateritia*) Whole grain



j: details of exine showing irregular, smaller muri

Figure 6 contd.

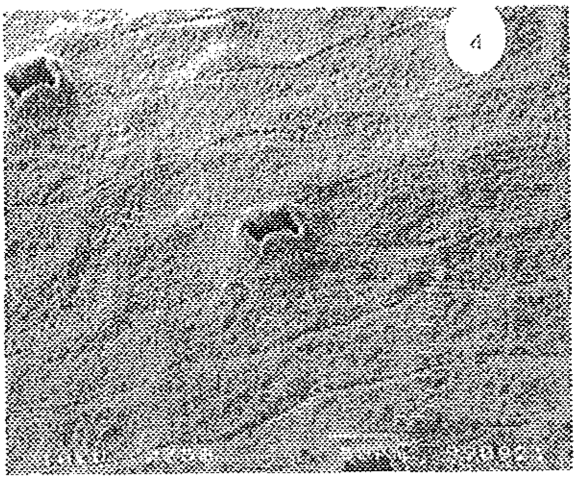
3.2.2 Cuticular features

As observed under x100 magnification, cells were 5-6 sided (fig.7a) with stomata pores sunken and arranged in more or less regularly parallel rows. At magnification of x1000, the presence of wax was evident. In some specimens, the wax presented as flaky substances while some specimens had dense masses of wax deposits. Cell walls were markedly unsculptured while the cell areas had outlines of micropappillae of variable density. A pair of guard cells, one guard cell conspicuously convex-lipped marked the stoamtal cavity (fig. 7c).

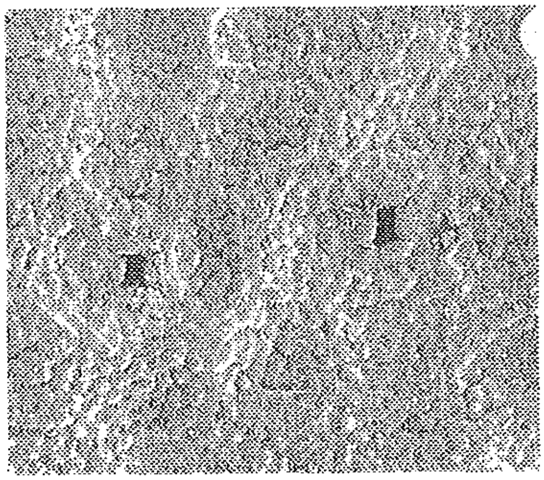
Specimens from Nanyuki and Gilgil (*A. lateritia* var. *graminicola*) had distinct epidermal cell pattern due to presence of regular anticlinal walls. Epidermal cells were 5-sided. The cell walls were unsculptured while the cell areas were criss-crossed by micropappilae that formed a reticulate pattern. Guard cells were relatively thick lipped. Wax in both cases was visible as upright flakes.

Epidermal cells in specimens from Gilgil (Fig. 7e) were more elongated than those from Nanyuki (Fig. 7d). Densities of micropappillae and of wax were higher than those exhibited in plants from the latter population.

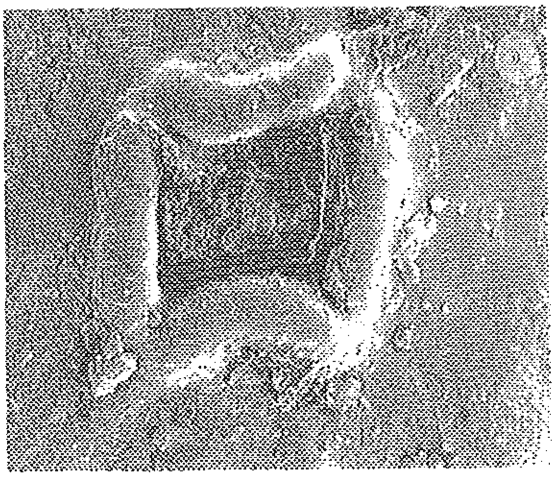
The regular 5-6 sided structures of cells were less distinct in specimens from Oloitokitok (*A. lateritia* var. *lateritia*) (Fig. 7f). This irregular cell pattern was crowned with haphazard arrangement of stomata. The cell outline was less distinct than that seen in populations of *A. lateritia* var. *graminicola* with much more packing of micropappillae in the cell areas. The reticulate pattern as seen in the former populations was evident but with denser networking. Outline of epidermal cells in specimens of *Aloe macrocarpa* (7g-h) was blurred, resulting in a pattern that was both indistinct and irregular with stomata haphazardly arranged. Cell areas were lined with scattered micropappillae. The reticulate pattern of microppapillae observed in the other specimens was exhibited but the surface deviated from those observed in other specimens in having more rectangular wax masses. The guard cells were also observed to be thinner-lipped than in populations of *Aloe lateritia*.



a-the regular 5-6 sides cell structure

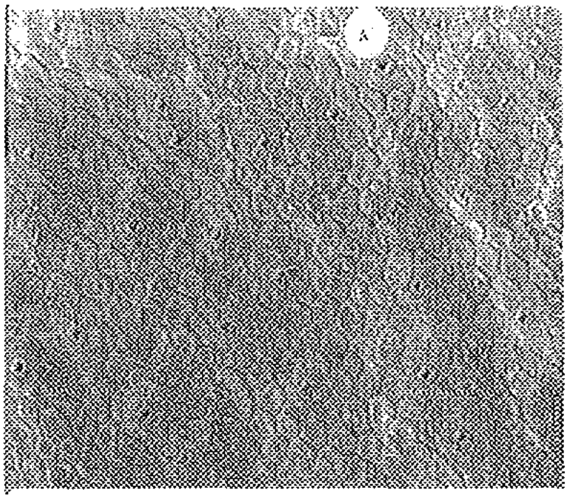


b: cell areas showing interlocking micropappilae and sunken stomata

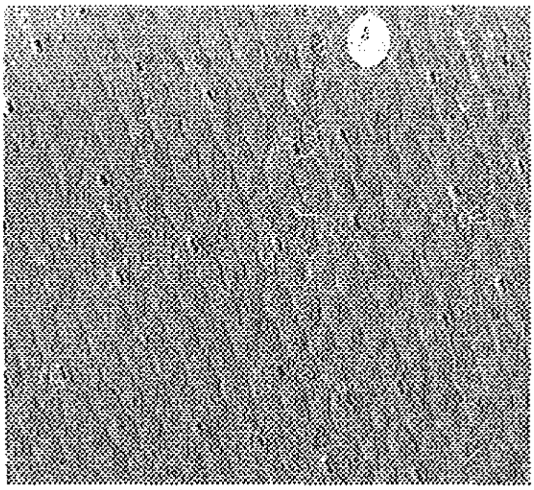


c: Asymetrical stoma showing a pair of convex lipped guard cells (lateral)

Figure 7: Cuticular Sculpture

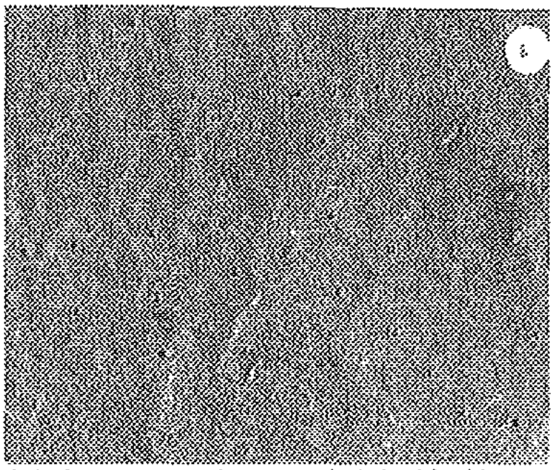


d: Nanyuki population



e: Gilgil population

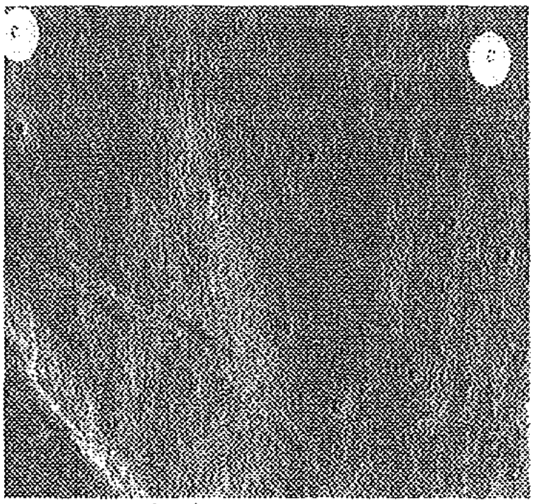
A. lateritia var. *graminicola* showing regular anticlinal walls, 2b more elongated cells than 2a



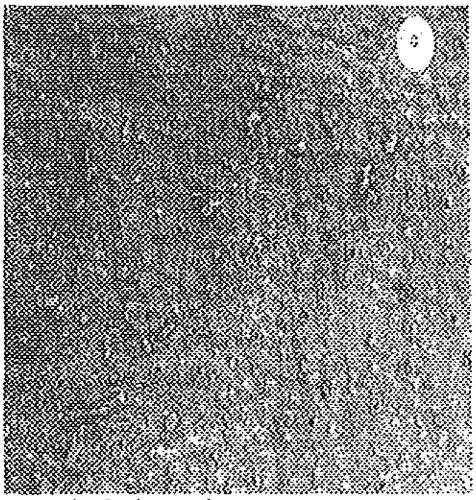
f *A. latritia* var. *lateritia* (Oloitokitok)

Showing the irregular cell structure and haphazard stomat arragement. Wax present as flakes

Figure 7 contd.



g: Shashemane



h Goha Tsion

A. macrocarpa showing the irregular cell structure (7g) and the regular wax masses (8h)

Figure 7 Contd.

3.3 Population studies

3.3.1 Background to the Study sites

As illustrated in figure 8, *A. macrocarpa* is found in Ethiopia, Sudan Cameroon, Nigeria and Benin. *A. lateritia* var. *lateritia* is found in both Kenya and Tanzania while A. *lateritia* var. *graminicola* has only been recorded in Kenya. For the present study, *A. macrocarpa* was studied at Shashemane and Goha Tsion, *A. lateritia* var. *graminicola* at Nanyuki and Gilgil and *A. lateritaia* at *Oloitokitok* respectively. The general ecological and geological characteristics of these regions are outlined below (Table 11).

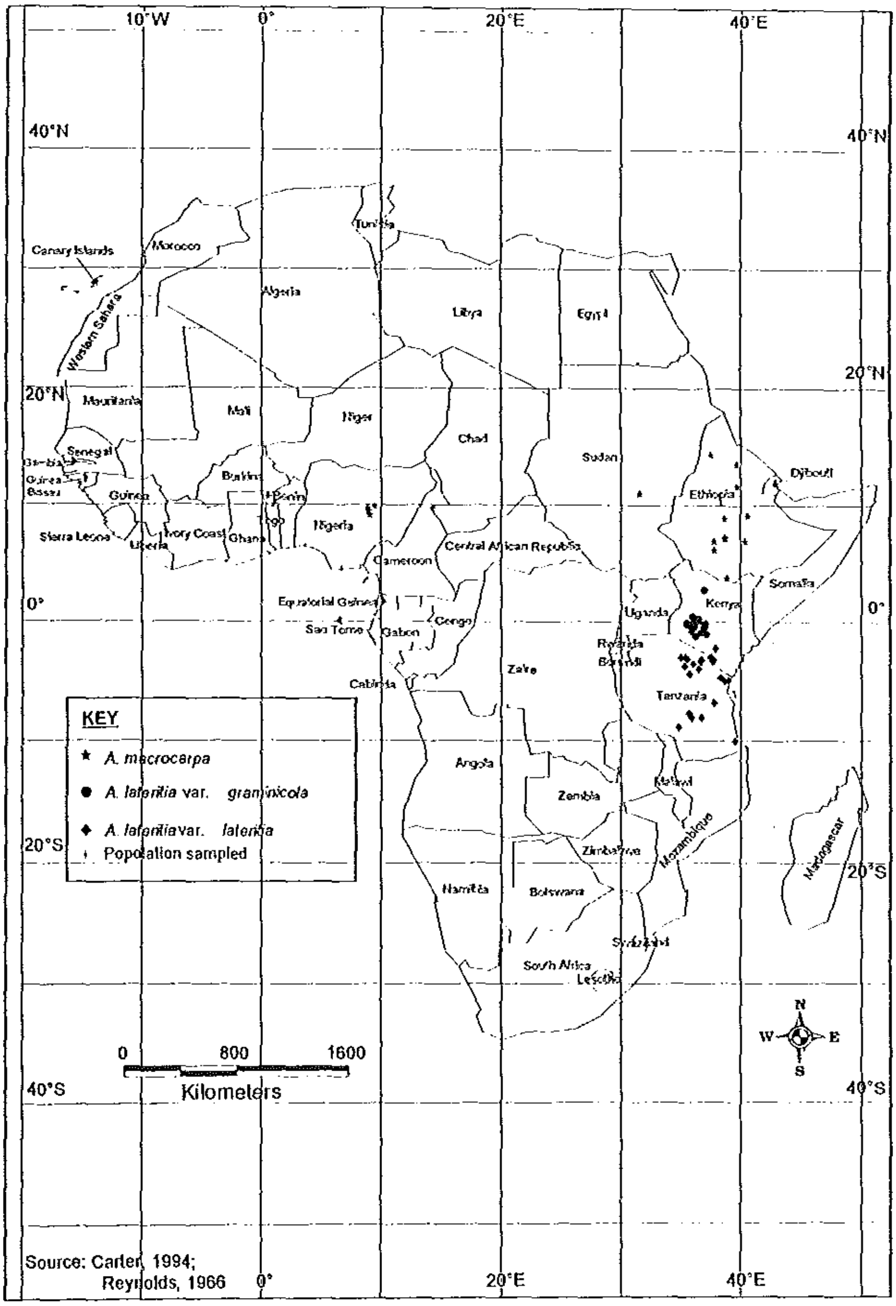


Figure 8: Distribution of *A. macrocarpa* and *A. lateritia*

Nanyuki Area- Kenya

The geology of Nanyuki area is described as being of middle and upper tertiary volcanic origin. The geological composition is mainly alkaline in nature consisting of basalts, phonolites, nephelinite, trachytes and alkali ryolites. The area is of marginal agricultural potential, carrying as natural vegetation dry forms of woodland and "savanna: (often an *Acacia –Themeda* association) or derived semi-evergreen or deciduous bushland. A range of soil types is found in the area most important of which are the brown calcareous loams (chestnut soils) and black clays.

Chestnut soils are derived from volcanic ash and show no profile development. They occur on plains between 1,200- 2000 m above seal level. The black clays include both calcareous and non-calcareous variants, usually derived from colluvial and occur on plains varying from very low altitudes to over 2000 m elevation. The Nanyuki area receives between 500- 600 mm of rainfall annually.

Nakuru-Gilgil Area

The geology of this area is composed of quaternary volcanics and sediments. These are of Pleistocene age, are dominantly alkaline in nature and are generally very similar to those of tertiary age. The quaternary deposits consist of thick deposits of lacustrine and flaviatile sediments among which diatomite beds are common. Like the Nanyuki area, land in Nakuru-Gilgil areas is of limited agricultural potential with woodland and "savanna" vegetation. The soils of this area are of recent lacustrine deposits, mainly river and lake sediments and have no developed morphology except a more humic surface. Lava boulders are a common feature of the landscape with pockets of dark clays and loams.

Oloitokitok area

The Oloitokitok area is of similar geological composition to the Nakuru- Gilgil area. It is composed of quaternary volcanics, which are mainly of alkaline composition. Land in Oloitokitok is only locally suited to agriculture. The vegetation is mainly woody with *Commiphora-Acacia* associations common. Perennial grasses like *Cenchrus ciliaris* and *Chloris roxburghiana* dominate the landscape.

The soils are generally classified as lithosols and regosols, including ash and pumice soils in some areas and black clays in others. Ash and pumice soils are dark-greyish brown humic (2% carbon) in nature and are derived from recent unconsolidated volcanic as on smooth rolling to level land at altitude between 600-2000 m above sea level. The " black cotton" soils include the calcareous and non-calcareous types at altitudes between 900 to 1,200 m elevations. The area receives between 400 to 1,100 mm of rainfall annually.

Goha Tsion area

This region is found in the central highlands and massifs of Ethiopia. The geology of the area is mainly basaltic rocks of tertiary (mainly lower tertiary) age. The rocks have a low content of SiO_2 and are therefore alkaline in nature. The vegetation is described as being dry evergreen montane type. The soils are rendzinas and haplic in nature with limited rooting depth. In this region, rendiznous (soils with only a mollic "A" horizon) rest directly on calcareous rock. They are of limited agricultural value because they generally occur on steep slopes. Many rock outcrops are found in the area. The mean annual rainfall in the area ranges from 400 to 799 mm.

Shashemane Area

This region is found at the floor of the rift valley and is categorized as belonging to the lower complex granite gneiss basement of Precambrian origin. The geology is composed of ignimbrites and pumice deposits. Volcanic ash is a common feature. The region is dominated by dry evergreen montane type of vegetation. Two types of soil occur in the region. These are chromic cambisol and eutric cambisol with a high content of SiO_2 making the soils acidic. The soils are of limited agricultural value. The mean annual rainfall is between 800-1179 mm.

Table 11: Physical and Ecological Background to the demographic sites

Locality/Zone	Altitude (m)	Geology	Soils	Vegetation	Temperature		Rainfall/ Seasonal max.	
					Min	Max	Short	Long
Nanyuki-Kenya	2103	Tertiary volcanics	Brown calcareous loams Black clays	Wooded grassland Grassland	6-10°	22-26°	Apr.	Nov.
Gilgil- Kenya	2100	Quaternary volcanics	Lava boulders Alluvium& lacustrine deposits Black clays	Wooded grassland Grassland	10-14°	26-30°	Apr.	Nov.
Oloitokitok- Kenya	1390	Quaternary volcanics	Ash & pumice Lava boulders Black clays	Wooded grassland/ Bushed grassland	14-18°	26-30°	Apri	Nov.
Shashemane-Ethiopia	1880	Precambrian formations	Sodic dark clays	Dry evergreen montane forest		15-20°C	Feb. & Jun.	Apr. & Aug
Goha Tsion- Ethiopia	2436	Tertiary volcanic (Basalts)	Dark clays	Dry evergreen montane forest	6-9.7°C	15-20°C	Mar. & Jun.	July

3.3.2 Demographic sites

Nanyuki Population (*Aloe lateritia* var. ***graminicola***)

This is the type population of *Aloe lateritia* var. *graminicola* It is located about 10 kilometers from Nanyuki town along the Nanyuki-Nairobi road. The locality is dominated by wooded grassland vegetation on gently sloping plains with black soils.

Plants at this site were found tucked under thick bushes, growing either singly or in clusters of up to 6 individuals. Most plants were acaulescent although short stems up to 50 cm were observed in a few isolated individuals. The leaves were growing in tight rosettes and were spotted at various intensities. In some plants spotting was heavier on the adaxial than on the abaxial surfaces while others were completely unspotted. The spotting formed irregular transverse bands. Shaded plants were observed to be light green in colour while plants that were exposed to the sun tended to acquire maroon colouration.

In a plot of 5 x 20 m^2, total of 39 genets and 47 ramets were recorded (1:1.2 ratio). The number of ramets per genet ranged between 1-5 (Fig.6a).

Pennisetum sp (Gramineae) was the dominant grass at the site. Other plants found at the site included *Euclea divinorum* (Ebenaceae), *Acacia drepanalobium* (Fabaceae), *Dodonaea angustifolia* (Sapindaceae), *Scutia myrtina* (Rhamnaceae), *Kalancloe lanceolata* (Crassulaceae), *Rhus natalensis* (Anarcardiacae) and *Solanum incanum* (Solanaceae).

At the time of the visit(s), signs of human disturbance and encroachment were evident. For instance, the plot in which sampling was done had been fenced off presumably in readiness for construction of residential houses. There were signs of harvesting of leaves, a fact that was confirmed by the local people.

Gilgil Population (***Aloe lateritia*** **var.** ***graminicola***)

This population is situated over 250 kilometers from the Nanyuki population. It is continuous "natural plantation" of aloes that covers close to 20 km^2 with several pockets of plants in the neighbourhood. The site is dominated by open grassland and occasional scrub. The terrain is dominantly flat plain with black soils.

At the time of the visit tall grasses dominated the site, shading the ramets from direct sunlight. Plants at this site were growing in thicker clusters than those in Nanyuki. Both acaulescent and caulescent plants were seen, the latter more prevalent in solitary individuals. Leaves were growing in compact rosettes, with spotting of different intensities. As observed in the Nanyuki population, some leaves had heavier spotting on the abaxial than on the adaxial, while others were completely unspotted. Leaf colour ranged from very pale green is shaded plants to reddish-green in the more exposed plants.

In the 20 x 5 m^2 plot mapped out, a total of 29 genets and 338 Ramets were recorded (91:11.6) (Fig. 6b). The number of ramets in every genet ranged from 1-24.

This population is relatively undisturbed in comparison with that at Nanyuki, probably due to the fact that the ground on which it stands is unsuitable for agriculture. The only signs of disturbance may be due to trampling by herds of zebra.

Oloitokitok Population (***Aloe lateritia*** **var.** ***lateritia***)

This is the only population of *Aloe lateritia* var. *lateritia* that was studied. It was a small isolated population with only 18 plants seen within a radius of 1 kilometer. Tufts of open grasses with dead shrubs dominate the habitat. Soils were of 'black cotton' type, on a dominantly flat plain landscape.

The plants were acaulscent, forming rosettes that were denser and stouter than those of recorded in the two populations of *A. lateritia* var. *graminicola.* The leaves were heavily spotted on both the adaxial and abaxial surfaces, the spots being regularly oval to round. The spotting formed regular transverse patterns, more pronounced on the adaxial surfaces. Some leaves had a purple tinge. Three genets and 18 ramets were recorded in the entire population. The number of ramets per genet ranged from 3-9 (Fig. 9c).

This population is located very close to the main road within a small market center. It is thus unlikely that it will be left undisturbed with increased development as well as harvesting by the local people who use it for medicinal purposes.

Shashemane Population (*Aloe macrocarpa*)

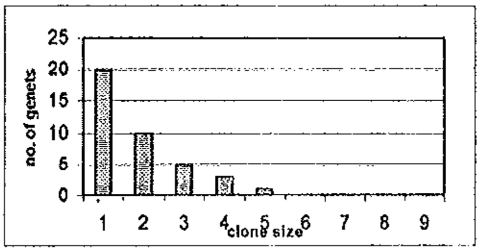
This population is found on eroded rocky slopes by the banks of the Dedeba River. The habitat consists of tufts of grasses with riverine vegetation in black soils. *Aloe pubescens* is common at the locality. The 20 x 5 m^2 of plot mapped out consisted of 34 genets and 62 ramets (1:1.82). The number of ramets per genet ranged from 1-5, with the largest cluster containing the largest plants (Fig.6d).

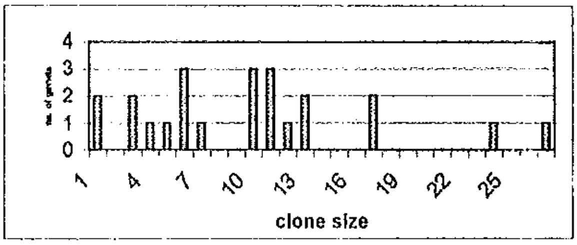
Goha Tsion Population

This was a small population consisting about 30 plants in total. It was situated on rocky slopes, at the side of a dry river valley. The vegetation at the site is composed of dry grasses and scrub, with most of the smaller plants being of succulent nature eg *Caralluma sp.*

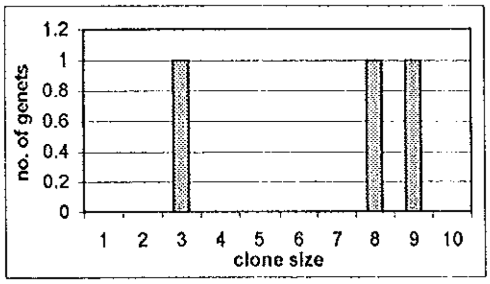
This population is situated on land that gently slopes towards the river gorge. No farming activities take place on this and the neighbouring land. However, small-scale quarrying takes place at the site a fact that jeopardizes the survival of the population. The presence of other species of *Aloe sp.* such as *A. debrana* may also limit population expansion due to inter-specific competition.

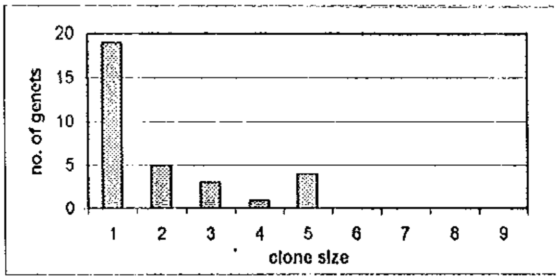
The entire population was made up of 21 genets and 32 ramets (1:1.52). The number of ramets per genet ranged between 1-4, with the densest cluster containing the largest (oldest) plants (Fig. 9e)



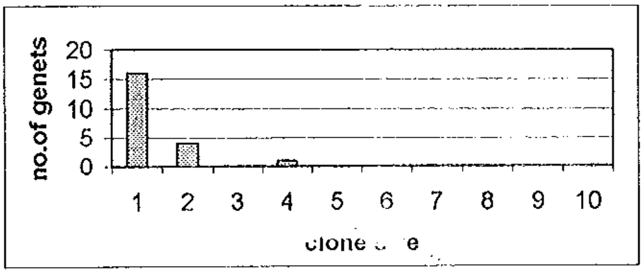


a- Nanyuki: *A. lateritia* var. *graminicola* b- Gilgil: *A. lateritia* var. *graminicola*





c Oloitokitok: *A. lateritia* var. *lateritia* d- Shashemane: *A. macrocarpa*



e: Goha Tsion- *A. macrocarpa*

Figure 9: The spatial structure of the 5 populations by clone size (number of ramets per **genet)**

Apart from observations made of the aggregation structure (clone size) of plants in the various populations, it was also noted that differences existed in the sizes at the sites. Figure 7 below shows the age composition of the various populations in which the total number of leaves on a plant was assumed to be directly related to the age of the plants.

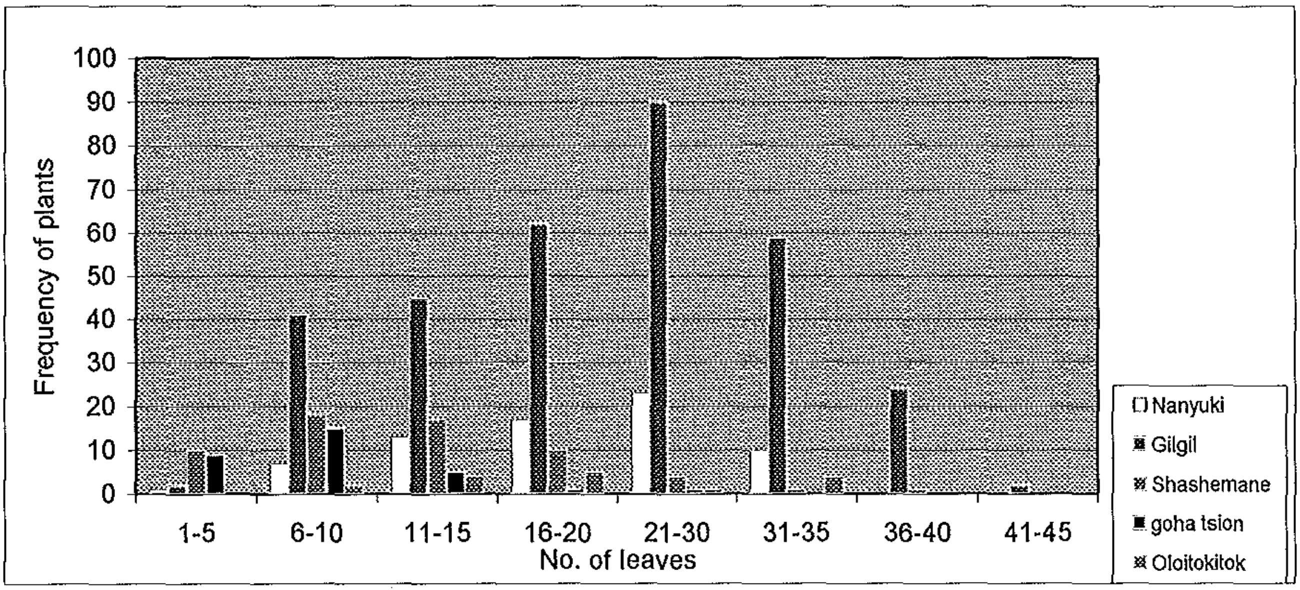


Figure 10: Structure of populations 1-5 by size of plants (Leaf Number)

The Oloitokitok population was shown to consist of predominantly medium-sized plants. Both small plants (1-5 class) and large ones (26-30, 30-35 & 35-40 classes) were un-represented. The Nanyuki population had very few small plants in the 1-5 size class (seedling stage) and none in the largest size classes (30-35 & 35-40). There was however evidence that the population is actively growing and recruiting new individuals since the intermediate size classes had a distribution that was almost normal.

The Gilgil population (*A. lateritia* var. *garminicola*) had all size classes represented, with the 21-

25 leaves size class having the highest number of individuals. The size structure as expressed in the histogram indicates that this is a stable population that stands high chances of future survival. There is no evidence of lack of recruitment or senescence. This is evidenced by the high rate of flowering and fruit set that were observed during the study period.

Both populations of *A. macrocarpa* consisted of a large number of small plants in the 1-5 leaves size class (seedlings). The Goha Tsion population seems to be predominantly youthful, with no individual in the large size classes. It is made up of individuals below the reproductive size. None of the plants flowered during the study period.

The Shashemane population was found to be made up of actively growing individuals, with many seedlings and medium-sized plants. The large size classes however, had increasingly less representation and no old (post reproduction) individuals were observed. A few plants flowered during the study period.

3.4 Population Dynamics

Data on population flux was obtained for only three out of the five populations initially mapped out for study. A summary of the data is presented (Table 12). The populations at Goha Tsion (*A. macrocarpa*) and Oloitokitok (*A. lateritia* var. *lateritia*) had been destroyed during the second visit. Thus, no measurements could be made for comparison with those taken during the first season of the study.

Table 12: Summarized population flux for the three population

Population	No. of genets	Total no. of modules	Births		Deaths
			Genets	Ramets	
Nanyuki	39	47	2	6	2
Gilgil	29	338	6	33	12
Shashemane	34	62	2	4	2

In all the three populations, it was observed that vegetative recruitment was much higher than recruitment of new genetic individuals. This was calculated as the number of new genets divided by the total number of modules recorded during the first season of study.

During the study period, recruitment by seed in the Nanyuki, Gilgil and Shashemane populations was 4.25%, 1.77% & 3.25% respectively. Vegetative recruitment (number of new ramets divided by total number of modules in season 1 of the study) was 15.5%, 9.7% and 6.45% respectively.

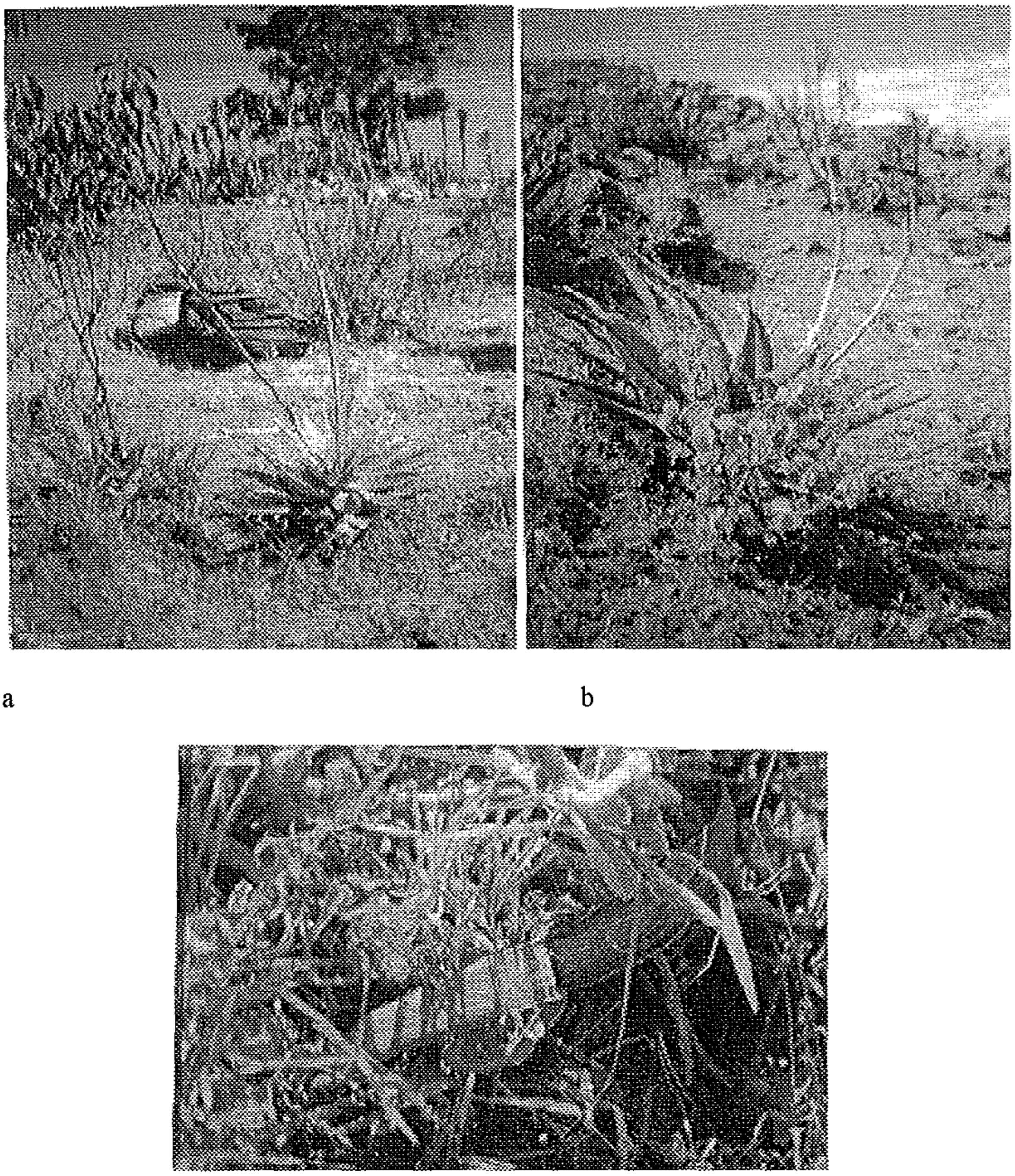
The amount of deaths recorded over the study period was equal to the amount of recruitment by seed in both the Shashemane and the Nanyuki populations. The Gilgil population recorded the highest number of deaths (3.55%). This could be attributed to the higher number of old individuals present in the population as opposed to the Shashemane and Nanyuki populations, which were more youthful.

3.4.1 Habitat Preferences and Associated Species

Aloe macrocarpa grows at altitudes between 1420 to 2200(-3000) m above seal level, in either semi arid or montane vegetation types (Gilbert & Sebsebe, 1997). In Quiha area (2200 m, 13° 30'N 39°35'E) of Ethiopia, the population is found growing on limestone rocks on dry overgrazed hillsides. This region receives up to 700 mm of rainfall per annum. The species has also been reported to occur Adaba and Bekoji (Ethiopia) on stony, gently sloping grassland (3000 m altitude) and in woodland in Fendika (Ethiopia) area at an altitude of 2060 m.

In its range of habitat, the species is associated with various other hardy plants. These include *Calpurnia aurea, Senna sp.*, Acacia *mellifera* (Leguminosae), *Croton macrostachyus, Acalypha fruiticosa Euphorbia borenensis* (Euphorbiaceae) *Ficus sp.* (Moraceae), *Combretum* sp.(Combretaceae), *Cadaba farinosa (Capparidaceae)*, *Aloe ottalensis* & *Aloe pubescens* (Aloaceae) *Cissus quadrangularis* (Vitaceae), *Sansevieria* (Agavaceae), *Plectranthus sp.* (Lamaceae), *Kleinia sp.* (Asteracae) and *Lintonia nutans* (Graminaeae) among others.

In the two populations studied (Shashemane & Goha Tsion), (Fig.11) the species was found growing on at the margins of small river gorges in rock strewn dark clays. In West Africa, the species is found growing on rocky hills and cliffs in association with other plants like *Fadogia sp.* (Rubiaceae)



a b

c

Figure 11: ***A. macrocarpa*** **(a) at the shasemane site on a gently slope by the river bank (b)** ***A. debrana*** **at the Goha Tsion site of** ***A. macrocarpa*****, on gentle slope with rock outcrops & (c) seedling of** ***A. macrocarpa*** **tucked under bushes at Goha Tsion site.**

Aloe lateritia var. *graminicola* grows at altitudes of 1420-2200m in the semi-arid areas of Kenya.

In most of its range, the species is a dominantly grassland dweller. This is true for the populations that occupy the plains between Naivasha (altitude 2100 m) and Nakuru (altitude 1767 m). However, it also occurs in the wooded grassland in Nanyuki (altitude 2100 m). The taxon occurs between altitudes of 1625 and 2530 m. It grows in 'black cotton soils and at its various sites is associated with other xerophytes like *Acacia seyal*, (Leguminosae) *Aspilia semota*, *Brachylaena huillensis*, (Asteraceae) *Dodonaea angustifolia*, (Sapindaceae) *Sarcostemma sp.* (Asclepiadaceae) and *Rhus natalensis* (Anacardiaceae) *Acacia drepanolobium*, *kalanchoe lanceolata* (Crassulaceae) *and Pennisetum sp.*

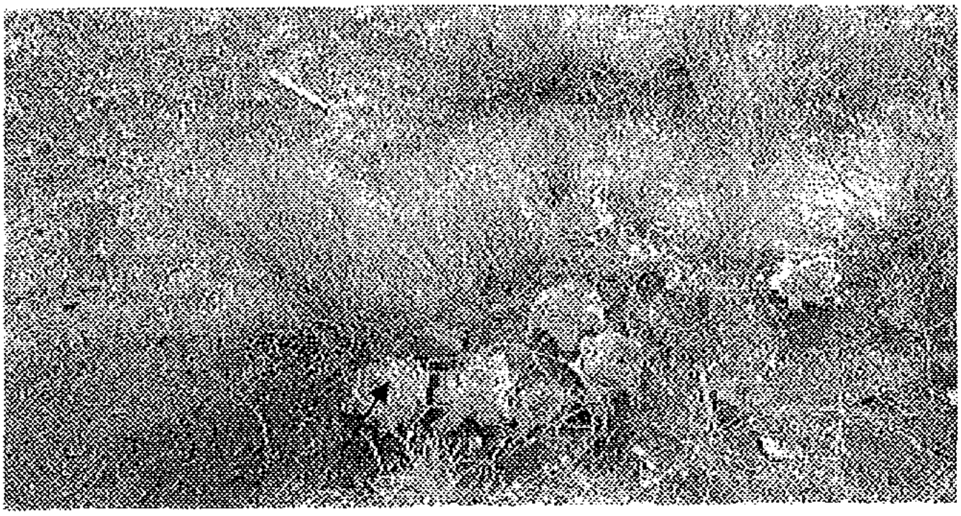
a- Nanyuki, b Gilgil

Figure 12: *A. lateritia* var. *graminicola* (a) showing intense variagation and maroon colour (b) showing green colour and variagation and (c) showing the habitat at Gilgil site, Plants in clusters

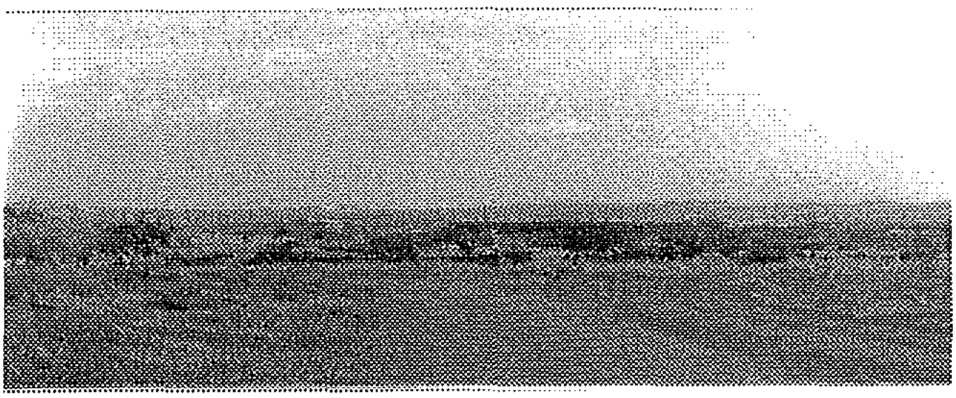
c- Gilgil

Figure 12 contind.

Aloe lateritia var *lateritia* occurs between 250 to 2125 m in open grassland or wooded grassland vegetation. It has been reported to grow at altitude as low as 70 m elevation in Tanzania (Harris & Pocs 4451 deposited at EA). It grows in rocky black clay soils and is frequently associated with other dry land species that include *Hibiscus sp.* (Malvaceae) *Aloe volkensii* (Aloaceae),*Acacia sp.*,(Leguminosae) *Brchystegia sp.* (Caesalpinaceae) and *Pericopsis sp.*. In Mbagala area of Tanzania it is frequently found growing on termite mounts in grey clayey soils.



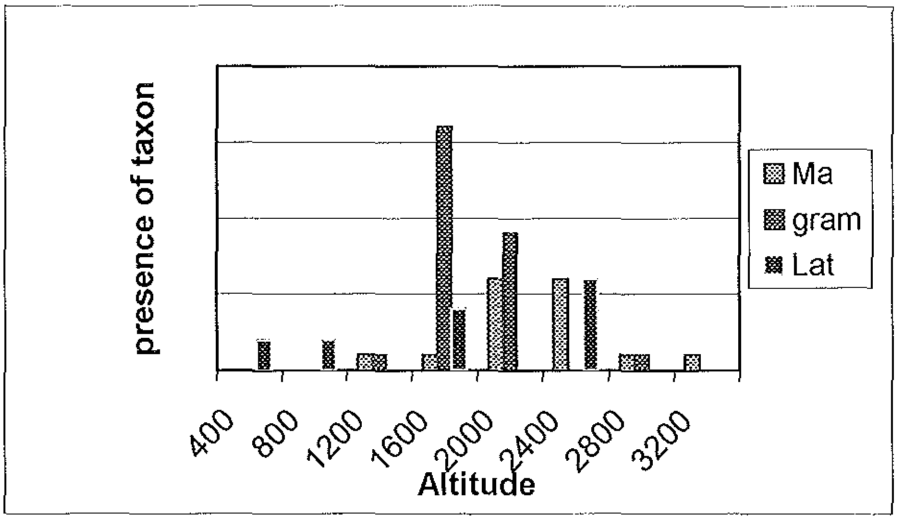
a



b

Figure 13: *A. lateritia* var *lateritia* (a) showing the cut stumps in the foreground and (b) the habitat at the roadsides.

the highest. However, there were overlaps in altitudinal ranges for the taxa..Figure 14).



Ma= A. macrocrapa gram= A. lateritia var. graminicola lat= A. lateritia var. *lateritia*

Figure 14: Altitudinal range for the taxa

3.3.2 Other information

The plants were known by different names by the people in the various localities visited. These names in most cases were used in reference to the species under study and all other *Aloe* species. In Nakuru (Kenya) and the surrounding areas the plants were known by the Kalenjin people as "Osuguroi" who, it was alleged used the plants in treating ailments of chicken. The leaves were chopped and mixed with water for the chicken diseases. In Nanyuki (Kenya), the plants are used by the local people for hastening the fermentation of traditional beer.

The local people in Oloitokitok claimed to use the plant (*A. lateritia* var. *lateritia*) in curing a cross section of human and livestock diseases. In Ololun'ga (Kenya) area, the plants have been reported to be used in hastening fermentation of beer. In Tanzania (Moshi) the leaves of the plants are pounded and given to patients for deworming.

curing a cross section of human and livestock diseases. In Ololun'ga (Kenya) area, the plants have been reported to be used in hastening fermentation of beer. In Tanzania (Moshi) the leaves of the plants are pounded and given to patients for deworming.
In Ethiopia, *A. macrocarpa* is known by the local name 'Ret" (Amharic) and Argesa (Oromifa) and is used for a range of purposes, from treatment of human ailments like malaria to personal hygiene as hair "shampoo".

3.4 Isoenzyme Analysis

The banding patterns (genetic phenotypes) presented in this section have a theoretical genetic basis to their interpretation, which includes the nature of enzyme assayed.

Phosphoglucomutase Enyme System (PGM) (Fig 15c)

Phosphoglucomutase is a monmeric enzyme; meaning that, homozygous genotypes will display single bands while heterozygotes will display two bands (each electromorph from either parent). In the present study, enzyme activity was observed in two areas. In the most anodal area, two to three bands were expressed, indicating that two loci are involved. In the three-banded region, the middle band was fixed. It was thus interpreted as a monomorphic locus and denoted PGM-2, a locus overlapping with PGM-1 with one allele (a) in front and another allele (b) at the rear.

In the most cathodal area, 1 to 2 bands were expressed. This indicates that only one locus (PGM-3) is involved. Banding was at 4 levels, interpreted as allele a, b, c and d respectively. Allele "c" was common to all populations while allele "a" occurred only in homozygous condition. Alleles "d" and "b" occurred only in heterozygotes.

displayed 3 to 5 bands. In view of the theoretical background, it is impossible to give a logical explanation to this peculiarity in terms of loci and alleles. It was decided to present the findings in terms of band phenotypes rather than genotypes.

Seven levels of bands were displayed in total, one of them always coinciding with and just in front of the most cathodal being consistently weaker. This band was accordingly interpreted as a ghost band and omitted in subsequent mapping (ghost bands have often been reported in the same position with respect to this enzyme (Nordal, Personal communication)

The rest of the bands were designated a(1a), b(1b), c(2a), d(2b), e(2c) and f(2d) respectively starting from the anodal towards the cathodal end of the gel and individuals scored. The a- band phenotype was common to all populations studied. Populations A and B displayed some deviating bands, a-e-f, a-d-f and a-b-f. Populations C and D expressed a-d-e, a-c-d-e, a-c-d-e-f and d-e-f as deviating band phenotypes. Population E displayed only the common a-f phenotype.

Catalase (cat) Glucose-6 Phospahate enzyme system (GPI) Fig 15a

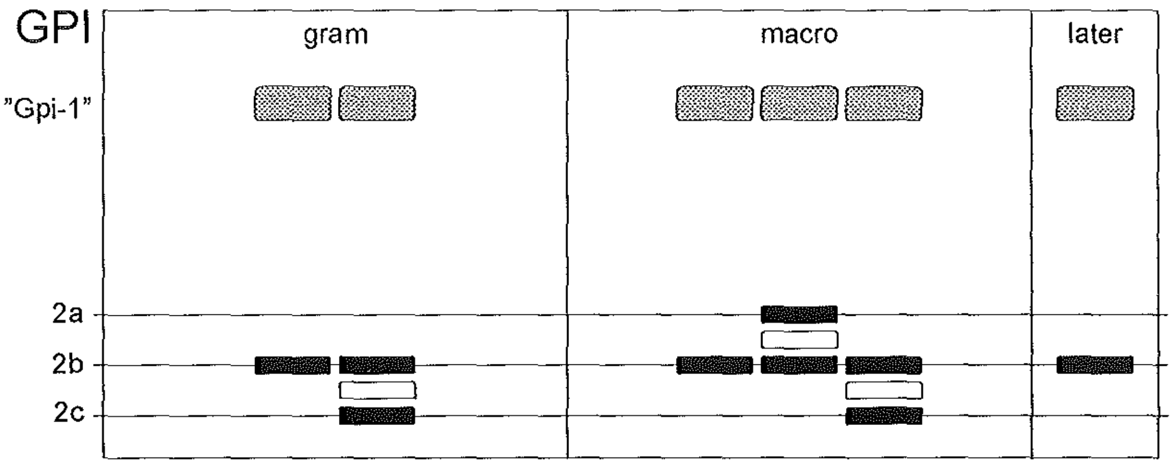
Catalase Glucose- 6 Phosphate is dimeric, in which case homozygous and heterozygous loci are expected to express single bands and three bands respectively. In this study, activity in the gels was noted in two areas. The most anodal one-denoted GPI-1.

The second area denoted GPI-2 displayed three allelic bands a, b and c respectively. The "a" allele was found in three individuals of population C and one of population D. Allele "b" was common in all populations sampled and was fixed in population E while allele the "c" allele was found in both populations B and D and not in population E.

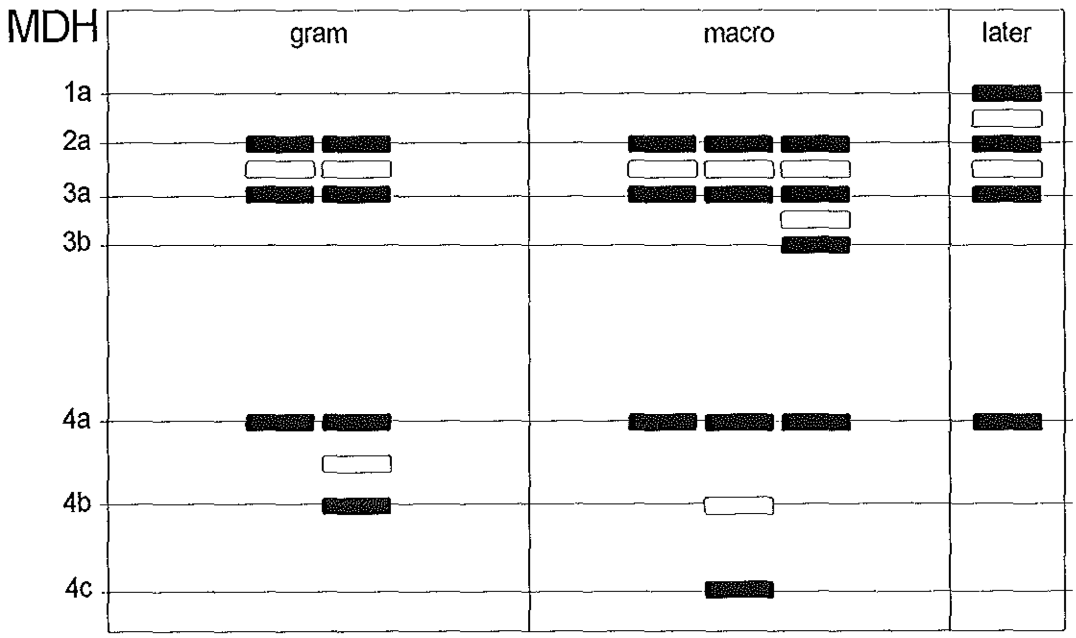
Malate Dehydrogenase enyme system (MDH) Fig. 15b

Malate Dehydrogenase is a dimeric enzyme. It is thus expected that homozygous loci would express single bands while the heterozygous ones would express 3 bands. In this study, activity was displayed in two areas. The anodal area had 3 to 5 bands, meaning that there must be more than one loci involved. Population E showed a constant 5-banded pattern interpreted as 3 loci (MDH-1, MDH-2 and MDH-3) with two inter-locus heterodimers. MDH-1 was not expressed in any other population. This means that it may represent a unique duplication in population E. Population A, B and C were monomorphic in MDH-2 and MDH-3. Population D displayed two alleles in MDH-3 ("a" and "b", the latter only found in heterozygote condition). MDH-4 displayed three alleles all together, allele "a" being very common, allele "b" only found in population A and allele C only found in population D.

- No enzyme activity was observed when the rest of the enyme systems (AAT, IDH & 6PGD) were stained.

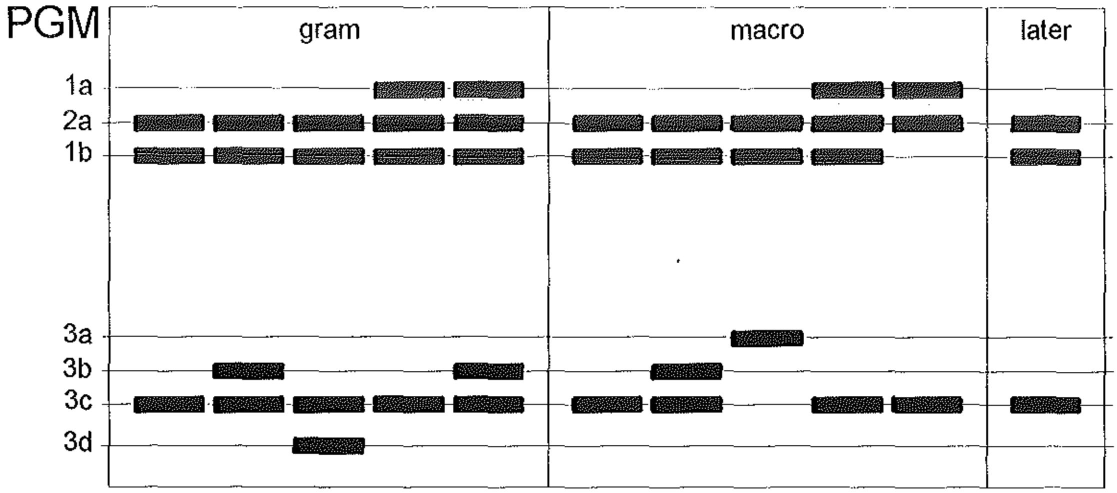


GPI enzyme system

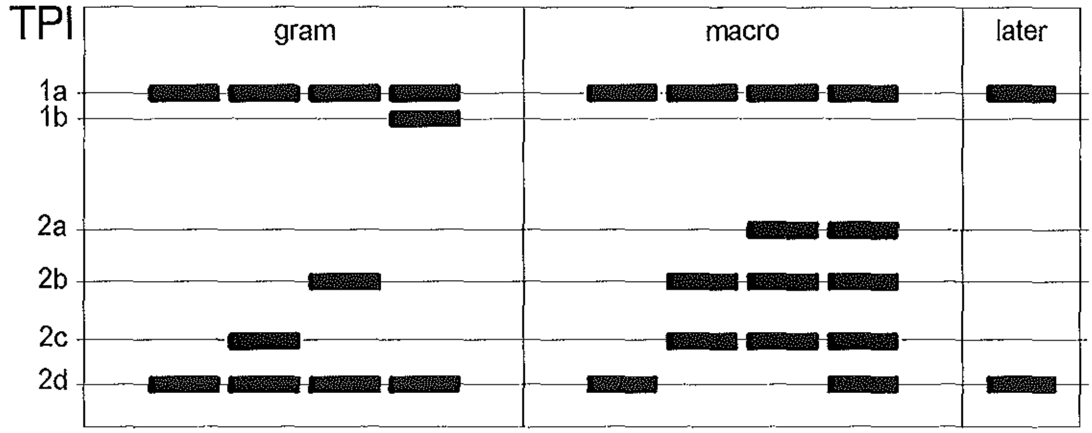


b Malate dehrdrogenase enyme system

Figure 15: Isoenzyme banding patterns for the 5 populations studied. a. catalase (cat) Glucose-6-phospahte (GPI) b. Malate Dehydrogenase (MDH) c. Phosphoglucomutase (PGM) and d Trisephospahate isomerase (TPI) enzyme systems.

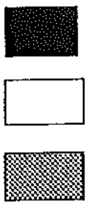


C Phosphoglucomutase enzyme system



d Triosephosphate isomerase enyme system

Legend



Allelic bands

Heterodimers

Blurred/ Ghost bands

macro: *Aloe macrocarpa* (populations C & D)

Gram: *Aloe lateritia* var. *graminicola* (Populations A & B)

Later:*Aloe lateritia* var.*lateritia* (Population E)

- Letters denote different alleles, numbered from the cathodal towards the anodal ends of the gels

Figure 15 contd.

Data obtained form band phenotypes (relative mobility of enzymes) was analyzed using SPSS software. Figure 16 below shows the clustering pattern of individuals from each of the five populations used in the electrophoresis.

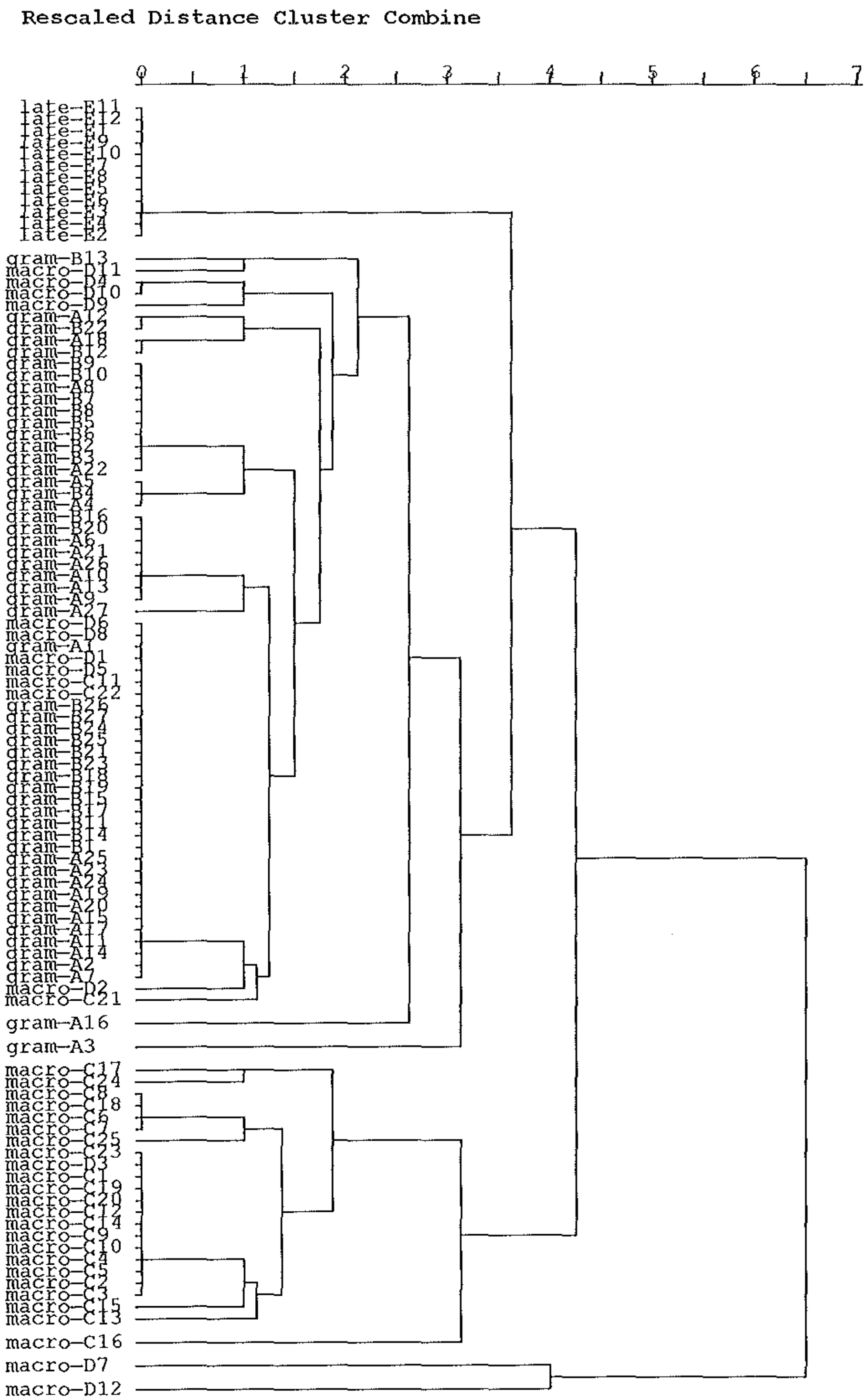


Fig 16: Phenetic structure of the 5 populations based isoenzyme banding patterns

3.5 Variation within populations

Observed heterozygosities (the mean expected heterozygosity assuming Hardy Weinburg equilibrium) were higher than expected in all populations except in population E (Oloitokitok) where both H and H_e were zero. The highest heterozygosity (H=0.1667) was observed in Population D (Goha Tsion) while the lowest (H=0.0880) was observed in population C (Shashemane). The trend in other parameters was similar to that observed in heterozygosity (Table 13).

Using the algorithm by Levene (1949) it was found that all the population were in Hardy Weinberg equilibrium

Intrapopulation variability value (Nei & Shannon indices) was highest in *A. macrocarpa* population (Goha Tsion) and lowest in the *A. lateritia* var. *laterirtia* (Oloitokitok).

Table 13:Genetic variation in populations of *A. macrocarpa* and *A. lateritia*

Population	P	Na	Ne	I	Nei	H	H_e
Nanyuki	80	2	1.1237	0.1938	0.0999	0.1111	0.1017
Gilgil	40	1.4	1.1286	0.1558	0.0934	0.1120	0.0953
Shashemane	60	1.6	1.0971	0.1460	0.0786	0.0880	0.0802
Goha Tsion	80	2	1.1864	0.28	0.1500	0.1667	0.1565
Oloitokitok	0	1	1	0	0	0.0000	0.000

P= % of polymorhic loci **Na**= observed number of alleles **Ne** = Number of effective alleles,

I= Shannon diversity Index **Nei**= Nei's diversity index **H_e**=Expected heterozygosity **H**= observed heterozygosity

Table 14 a Nei's Original measures of Genetic Identity (above diagonal) and Distance (below diagonal)

Population	Nanyuki	Gilgil	Shashemane	Goha Tsion	Oloitokitok
Nanyuki	****	0.9971	0.9961	0.9878	0.9955
Gilgil	0.0029	****	0.9973	0.9843	0.9915
Shashemane	0.0039	0.0027	****	0.9914	0.9957
Goha Tsion	0.0123	0.0159	0.0087	****	0.9943
Oloitokitok	0.0046	0.0086	0.0043	0.0058	****

Table 14 b: Nei's Unbiased Measures of Genetic Identity and genetic Distance: (Identity above diagonal) and genetic distance (below diagonal).

Population	Nanyuki	Gilgil	Shashemane	Goha Tsion	Oloitokitok
Nanyuki	****	0.9982	0.9971	0.9902	0.9960
Gilgil	0.0018	****	0.9983	0.9866	0.9920
Shashemane	0.0029	0.0017	****	0.9937	0.9962
Goha Tsion	0.0099	0.0135	0.0063	****	0.9962
Oloitokitok	0.0040	0.0081	0.0038	0.0039	****

Genetic distance values ranged from 0.0027 (Shashemane & Gilgil) to 0.0123 between (Goha Tsion & Nanyuki). The trend in genetic similarity was similar with the greatest similarity shown between the two populations of *Aloe lateritia* var. *graminicola* (Nanyuki & Gilgil) and the least (0.9843) similarity between the Nanyuki (*A lateritia* var. *graminicola* and Goha Tsion (*A. macrocarpa*) populations.

Table 15 Summary of F-statistics and Gene Flow for all Loci-

Locus	Sample size	Fis	Fit	Fst
PGM-1	102	-0.1688	-0.0987	0.0600
PGM-3	102	-0.1146	-0.0481	0.0596
GPI-2	102	0.0112	-0.0578	0.0471
MDH-3	102	-0.1429	-0.0256	0.1026
MDH-4	102	-0.0771	-0.0176	0.0552
Mean	**102**	**-0.1185**	**-0.0512**	**0.0601**

Fis= variation of individuals relative to the total population variation

Fit= Total variation of the population and its subpopulations

Fst= Total differentiation among populations

Wright's (1978) fixation Index quantifies inter-population differentiation. The mean F_{ST} value (0.0601) for polymorphic loci in the populations studied indicated low differentiation between the populations. Within population variability accounted for the remaining variation 0.9399 (94%).

4.0 Discussion

4.1 Evidence from External Morphology

In previous classifications (Reynolds, 1966, Carter, 1994), *Aloe macrocarpa, A. lateritia* var. *graminicola* and *A. lateritia* var. *lateritia* have been distinguished mainly on the basis of the raceme shape (length to width ratio), leaf size, density and length of marginal teeth and the size (diameter) of the perianth at the base and constriction. Thus, in the present study, apart from examining the gross morphological features of the taxa, emphasis was laid on these character states in an effort to discern the trends and any gaps that may confirm the validity in taxonomic diagnoses (table 8)

Leaf size

Although the averages in leaf sizes may differ slightly, it is clear that the nature of variation in leaf size is continuous, giving no gaps for species delimitation. Coupled with the fact that leaf size is heavily influenced by environmental factors, this character state was found unreliable in species diagnosis. However, the overlapping values of leaf size as obtained in this study suggest close relationship between the taxa.

Raceme Shape

Earlier studies have particularly emphasized the high occurrence of capitate racemes in *A. lateritia* var. *graminicola* as opposed to the longer (lax) racemes observed in its counterpart, *A. lateritia* var. *lateritia*. From observations both in the field and on herbarium specimens, it is true that there is a tendency in some populations towards one kind of raceme shape. However, it was observed that all the three raceme shapes are represented in the taxa. Of the specimens examined, the emerging trend in raceme shape was similar to that observed in earlier reviews (Reynolds 1966, Carter, 1994). However, the fact that all raceme shapes were represented in all taxa indicates overlaps (and therefore lack of gaps) that suggest the unreliability of raceme shape in separating the three taxa. It has been noted that raceme shape is heavily influenced by environmental variables (Heywood, 1963; Stace, 1989).

Marginal Teeth

Enumeration of marginal teeth (measured per five centimeters from the leaf base) shows a gap between *A.macrocarpa* on the one hand and the two subspecies of *A.lateritia* on the other. The range of measurements were 6-16, (3)5-11 and 5-11 in *A. macroacarpa*, *A. lateritia* var. *graminicola* and A. *lateritia* var. *lateritia* respectively. This evidence (density of teeth) could therefore be used to distinguish (only partially) between *A. macrocarpa* and its counterpart *A. lateritia*. The reliability of this character state is however uncertain since like the leaf size, it may be under heavy environmental influence.

Measurements of tooth size did no show any obvious discontinuity between the three taxa.

Perianth diameter

Reynolds (1966) observed that the basal swelling in *A. lateritia* var. *graminicola* is much narrower than in *A. lateritia* var *lateritia*. In the specimens examined the measurements ranged from 0.2- 0.8 cm (*A. lateritia* var. *graminicola*) to 0.4-0.6 (*A. lateritia* var *lateritia*) to 0.4-0.9 cm (*A. macroacrpa*). The overlap was also evident in the size of the perianth diameter at and above the constriction. No gaps were displayed between the taxa.

4.1.5 Overall Assessment of the reliability of gross morphology

Based on observations of representative specimens both in the herbaria and in the field, the elasticity of morphological characteristics in the studied taxa is extensive. Divergence from the typical forms as described in the various Flora abounds, with obvious similarities in adaptation to environmental heterogeneity. For instance, the range in colour and maculation of the leaves tends to follow a similar trend in all populations, with leaves in shaded microhabitats being pale green with fewer spots while those in exposed sites had various shades of colour between purple and maroon and increased spotting intensity.

All the other character states examined showed overlaps, thus suggesting continuity in variation. However, it would be inaccurate to make any taxonomic ruling on the position of the taxa studied until all the other evidences (cuticular sculpture, pollen morphology, population structure, isoenyme data) have been considered.

4.2 Evidence from Cuticular Structure

Cutler (1969) demonstrated that cuticular sculpturing in the members of the genus *Aloe* is characteristic for a given species, with closely related species having similar patterns, which can only be distinguished by closely examining all the cuticular components like wax, stomata and epidermal cell outlines. It was further noted that this sculpturing is under close genetic control which (within a clone), is little affected by environmental heterogeneity.

In the present study, all the specimens examined exhibited sunken stomata and wax covering both of which are of wide occurrence in the genus and in other xerophytes. The basic 5-6 sided cell structure observed earlier (Newton, 1972, Cutler, 1969) was also observed. In all the specimens examined, the basic sculpturing pattern was similar in having markedly unsculptured cell walls and cell areas having micropapillae. Close similarities were observed between specimens from Gilgil and Nakuru (*A. lateritia* var *graminicola*). However, specimens from Gilgil showed more elongated epidermal cell structure.

Specimens of *A. lateritia* var. *lateritia* displayed a less distinct cell (5-6-sided) pattern and stomatal arrangement was haphazard. This indistinctness in cell structure was amplified in specimens of *A. macrocarpa* (Goha Tsion & Shashemane). However, specimens of *A. macrocarpa* differed from those of *A. lateritia* var. *lateritia* in having wax deposits that were more or less rectangular in shape. The wax deposits in specimens of *Aloe lateritia* was in form of flakes.

Although there is evidence that epidermal surface sculpturing in *Aloe* species is genetically controlled, the influence of the environment cannot be eliminated altogether. For instance, cell turgidity and the condition of the stomata at the time of microscopy may have contributed to deviant cuticular features, especially in populations (specimens) of the same taxon. However, there is evidence that genetic differentiation exists between populations of the different taxa examined, the level of which remains uncertain.

This evidence suggests an order of both relationship and distance between sets of populations studied and is in support of the geographical locations of the populations i.e., close similarity is implied between populations of A. *macrocarpa* and those of *A. lateritia* var. *lateritia*. Specimens of *A. lateritia* var. *graminicola* tend to occupy an intermediate position between these extremes.

4.3 Evidence from pollen morphology

Stuessy (1994) noted that great variation exists in pollen features of most taxa. Erdtman (1969) recorded that variation in pollen feature at species level often has a cytological basis. Earlier studies by Erdtman (1969) and Steyn *et al.* (1998) indicated that *Aloe* pollen grains were monosulcate and have medium size (P.L=25-50µm)

The grains observed in the present study were large in size (Polar length=52.2-67µm) as opposed to earlier observations by Steyn *et al.* (1998). There were no deviations from the basic monosulcate nature. Further similarities were observed in the narrow variation of symmetry among and within populations from perprolate to prolate.

Similarities were expressed between the varieties of *A. lateritia* in that their specimens displayed lumina that were irregular. However, lumina in *A. lateritai* var *graminicola* were larger (0.82µm) than those of A. lateritia var. *lateritia* (0.42µm). The average lumina size showed similarity between *A. macrocarpa* (0.82µm) and *A. lateritia* var. *graminicola* (0.95µm) as opposed to the much smaller lumina observed in *A. lateritia* var. *lateritia* specimens. Differentiation in muri size amongst populations was minimal as it ranged from 0.42µm (*A. lateritia* var. *lateritia & A. macroacarpa respectively)* to 0.5µm in specimens of *A. lateritia* var. *graminicola.*

4.4 Evidence from Population Studies

4.4.1 Spatial Structure & Dynamics

The six months duration within which population studies were carried out was insufficient in providing accurate estimates of population parameters like births and deaths. The population data should therefore be evaluated on its usefulness for purposes of showing general trends and comparisons of the level of aggregation and modes of recruitment in the populations and not on its absolute accuracy in demonstrating demographic dynamism in the populations studied.

Hutchings (1997) noted that plant population structure is a product of interactive forces of both biotic and abiotic factors that shape individuals as well as the pressures that acted on their ancestors. Both the genetic structure and the performance of populations are shaped by these same factors.

As observed in the present study (figures 9) no uniformity in terms of clone size was expressed in the populations of the same taxa. Although the genet:ramet ratio in some of the populations were similar, the ratio in the two populations of *A. lateritia* var. *graminicola* are very different 1:1.2 & 1:11.6 respectively).

The genet to total ramet ratio in the population of *A. lateritia* var. *lateritia* was 1: 6. This trend may be evidence to the fact that the various populations have been subjected to different environmental pressures in the past, which have shaped their present structures. These may include human interference as observed in the Nanyuki and Goha Tsion sites. The Gilgil site is undisturbed and may have remained so for a long time with the only source of interference being herds of grazing zebras. This may explain its "population explosion" as compared to the other 4 populations.

Measurements of ramet height (RH) and ramet diameter (RD)were found unreliable in predicting the size (age) of the plants because the results showed that RH and RD in the plants studied (and aloes in general) could increase, decrease or remain fixed, depending on the availability of water.

This fact was attributed to their succulent nature. For instance, during the second visit to the population site (at the end of the dry season), most plants had reduced in size, while a few maintained the sizes as had been recorded in the first season. On the contrary, new leaves had emerged in many plants, thus making counts of leaf number a more reliable variable in population monitoring.

The spatial structure of the populations as observed in this study is of little use in species delineation. However, the similarity of the specific habitat occupied by the populations and the species that are associated with the various populations are useful hints to the ecological closeness of the populations sampled. The observed level of aggregation is useful in explaining the genetic structure as found from the isoenzyme patterns.

There is evidence to show the occurrence of higher vegetative recruitment in all the populations (Nanyuki=75%, Gilgil=84.61% & Shashemane=75%) as compared to recruitment of new genets. From the observations made in some of the populations (Shashemane, Nanyuki & Oloitokitok) the densest clones contained the largest plants. This trend was not observed in the Goha Tsion population, a fact that could be attributed to the fact that the population was generally young or may have been recovering from recent disturbance.

In the Gilgil population, it was observed that an optimum point exists up to which the average plant size increased with clone size. After this point, the plants tended to reduce in size, probably due to intra-specific competition for space and nutrients. Since large clone size increases the chances of persistence for individual genets, the Gilgil population was assumed to have the highest relative survival of all the populations sampled (Dessalegn, 1999).

These observations are in support of earlier findings by Stokes & Yeaton (1995) in their studies on populations of *Aloe candelabrum* in which they established that the species exhibited clumping in young populations, with clumps acting as nuclei from which plants spread slowly over time with mature individuals forming centers of the densest stands. As a result of limited seed dispersal intense competition resulted and consequently self-thinning within the stands.

4.4.1 Genetic Structure

The dendogram obtained form the isozyme data (Fig.16) shows the occurrence of three clusters in the five populations analyzed. Cluster 1 consisted of population E (Oloitokito- *A. lateritia* var. *lateritia*) only; cluster 2 is dominated by populations A (Nanyuki) and B (Gilgil) with a few of population C (Shashemane) and D (Goha Tsion); cluster 3 consisted of population C and D (*A. macrocarpa*). The cluster analysis suggests both similarity and distance in allozymic diversity in the populations studied. Cluster 1 is homogenous (probably due to lack of allozymic polymorphism or sample size limitations) and is closer to cluster 2 (population A & B) than it is to cluster 3, which exclusively consists of populations of *A. macrocarpa* (population C & D). It follows from the clustering pattern that, *A. macrcarpa* (Population C & D) and *A. lateritia* var. *latertia* occupy extreme positions (in terms of allozyme diversity) on the allozyme scale with populations of *A. lateritia* var. *graminicola* (population A & B) being intermediate, but nonetheless very similar to some individuals of *A. macrocarpa.* This accounts for the occurrence of individuals of populations C and D in cluster 2.

Hamrick *et al.* (1989) note that most plants maintain relatively high levels of variation within populations. In this study (Table 13), all the populations except that of Oloitokitok (*A. lateritai* var. *lateritia*) expressed more polymorphic loci than expected in an average plant species (37%) but was lower (Gilgil & Oloitokitok populations) than observed in other monocots (Hamrick *et al*., 1989). All the measures of genetic diversity indicate the highest variation to be found in the Goha Tsion population (*A. macrocarpa*) and the least in the Oloitokitok population. The number of alleles expressed was generally lower than that observed in other monocots (table 3a & 3b). Heterozygosity levels and other diversity measures (Nei & Shannon indices) were generally lower than for other monocots.

Speculative explanations of the low allozymic variation in *Aloe* species attribute the phenomenon to the xerophytic habit of the plants, which makes them less sensitive to drought stress that is the most important selection pressure in the dry lands (Van der Bank *et al*, 1995). Besides, it has generally been observed that populations, even of the same species have different genetic diversity. This was explained in terms of the life history and ecological characteristics of speciess (Hamrick *et al*. (1989).

In a biochemical analysis of the *A. candelabrum* and *A. ferox*, Viljoen *et al*. (1996) obtained value of 0.203, 0.465 and 0.574 for F_{ST}, F_{IS} and F_{IT} respectively. The low F_{ST} (0.203) value was interpreted as representing low levels of inter-populational differentiation, the low F_{IT} value (which quantifies inbreeding due to population subdivision) as not being indicative of effective barriers to gene flow between the populations studied and the low F_{IS} value as reflecting genetic drift (allelic fixation in the populations).

In the present study, the calculated values of F_{ST}, F_{IT} and F_{IS} (0.0601, -0.0512 & -0.1185) (table 15) are much lower than those obtained by Viljoen *et al*. (1996). It can therefore logically be concluded that the calculated F_{ST} value indicates extremely low inter-populational differentiation. Observations of F_{IS} and F_{IT} may however not accurately apply to the taxa studied since the populations may not be strictly panmictic (Kifle Dagne- personal communication)

Thorpe (1982) estimated values of genetic distance of less than 0.3 to be predictive of conspecific populations. The calculated values in the present study (Table 14a-b) were much smaller, strongly indicating that the observed genetic differentiation pertains to conspecific populations. Crawford (1989) noted that populations of the same species are very similar allozymatically, with mean genetic identities above 0.90.

4.5 Taxonomic Synthesis of the Evidences

Morphological data have illustrated the lack of discontinuity in the features previously used in separation of *A. macrocarpa* from *A. lateriatia* (*A. laterit*ia var. *graminicola* & *A. lateritia* var *lateritia)*. The overall shape, symmetry and form of pollen grains in the studied taxa indicates evolutionary or ecotypic closeness but the ultrastructure (SEM) suggests slight differences which could be synonymous to corresponding slight differences in the genetic differentiation between the studied populations. This hypothesis is supported by data from SEM studies of the leaf cuticle.

Genetic data have consistently shown low levels of differentiation among the populations studied. The low F_{ST} value is a pointer to possible gene flow between the populations and genetic distances that are far less than that considered being predictive of conspecific populations (0.3). The calculated identity values (Nei, 1972 Nei, 1978) in the present research is higher (table 14a-b).

From the genetic data, 2 evolutionary possibilities are implied. While it is possible that there is gene flow between the studied populations, the similarity in genetic composition may imply recent isolation of the populations from each such that the populations still retain the parental gene pool. This hypothesis cannot be tested on the basis of data collected in this research. On the other hand, it is possible that the similarity in gene pool is in response to similar selection pressures that are imposed by similar environments.

The second hypothesis in part plausible from the similarity in edaphic, ecological and geological conditions under which the populations of the taxa thrive, as well as the similarity in elastic responses (morphological) towards environmental conditions. For instance it was observed that in all the populations, the plants developed stems whenever they grew singly as opposed to their dominantly acaulscent nature when they grew in thick clusters. The second hypothesis is particularly plausible in view of the fact that genetic distance was highest between the Oloitokitok (*A. lateritia* var. *lateritia*) population and that of Goha Tsion (*A. macrocarpa),* a fact that could be attributed to the large differences in altitude, (Oloitokitok-1390 m & Goha Tsion-2435m respectively) which may consequently imposes its influence on other environmental variables.

According to Hedberg (1957) species are separated from each other by gaps of genetic discontinuity in morphological and physiological characteristics, which are maintained by the absence or rarity of gene interchange between members of different species. It is also noted that in nature, many cases where differentiation has not yet resulted into complete speciation are encountered.

Hedberg (1957) assigned subspecific rank to populations in which there existed complete discontinuous variation in at least one feature, preferably reinforced by partial discontinuity in other feature. The rank was also applied to regional subdivisions of a species. The status of 'variety' was assigned to populations of one or several biotypes that contained discontinuous variation in more or less conspicuous morphological features. Similarly, Sebsebe Demmisew (1985) assigned specific rank in cases where discontinuities existed in several morphological characters, and subspecific rank for geographical races

5.0 CONCLUSION

The absence of significant inter-populational differentiation has been shown from morphological observations and supported by all evidences undertaken in the present research. No single trait has shown complete discontinuity. The discontinuous variation in morphological traits among populations suggests subspecific status for the two taxa. Based on the above sources of evidences, I suggest suggest that *A. macrocarpa Todaro* and *A. lateritia* Engler are conspecific and therefore *A. lateritia Engl.* (1895) be considered a synonym of *A. macrocarpa* (1875) Tod.which is the earlier published name.

6.0 RECOMMENDATIONS

The present study pioneered specific studies on members of the series *saponariae* of the genus *Aloe* and focused on only one species complex in a single region. It is recommended that more extensive studies should be carried out on members of this group whose distribution is centered in southern Africa in order to obtain comparative data. These studies would be more complete if crossing studies could be carried out to observe the possibility of interbreeding within populations. Cytological studies are also recommended.

The succulent nature of *Aloe* species makes preparation of herbarium specimens a technical task, which is often not accomplished by many collectors. Future studies should aim to make more extensive measurements of fresh material to eliminate the bias introduced due to shrinkage of specimens.

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Appendix 1: Specimens Seen for the present study

Herbarium Code	Species	col. & no.	Region	Altitude	latdeg	latmin	N/S	longdeg.	longmin.	
ETH	**A. macrocarpa**	Burger, W. 3615	Assabut mts.	1300	9	8	N	40	35	E
		ermias & Sebsebe 2215	Ghinda							
ETH		Ensermu, Muhammed, Tamene, 2520	Shashemane	2000	7	14	N	38	40	E
ETH		Gilbert 1150	Adaba-Bekoji	3000	11	37	N	39	40	E
ETH		Gilbert &Phillips 8862	Arba Minch	1330	6	14	N	37	44	E
ETH		Gilbert, Ermias, Sebsebe,056863	De'deba River	1800	7	14	N	38	40	E
ETH		Gilbert, Phillips with Damtew 9265U	Gughe Highlands	2150						
K		Hall, JB c495	Nakpanduri							
K		Kea	Nigeria, Jos	9.5	0	N	9	0	W	
K		Latilo, MC	Nigeria, Jos	9.5	0	N	9	0	W	
ETH		Mooney 9597	Quiha,	2200	13	30	N	39	35	E
K		Reynolds 7241	Belgian Congo							
ETH		Sebsebe,Ermias,Steglich, 2380	Goha Tsion	2500	10	2	N	38	16	E
ETH		Sebsebe, Fikre, Ermias Dagne, Afework 4238	Shashamane	1970	7	14	N	38	40	E
ETH		Sebsebe, Mirutse & Tigist 5149	Fendika	2060						
ETH		Sebsebe 2426	Robe	2280	3	44	N			
ETH		Sebsebe 2558	Mega	1882	3	44	N	38	50	E
ETH		Sebsebe 2945	Daleti	2350	8	54	n	38	38	E
ETH		Sebsebe 5528	Addis Ababa (cultivated)	2400						
ETH		Sebsebe, Gilbert & Ermias 9304	Shashemane	1880	7	14	N	38	40	E
K		unknown	NE Cameroon	1100						
EA	**A. lateritia var. graminicola**	PRO Bally 10702								
		Bally 13786	Rumuruti	1500						
		PRO Bally 11424	Kijabe,		0	56	S	36	35	E
		Bally 8189	Nakuru	1767	0	15	S	35	55	E
		Brown, E.S. 1751	Hyrax Hill	1828						
		Christian H. B. 10895	Elburgon		0	16	S	35	36	E
		Davidse 7041	Naro Moru	1900	0	10	S	36	19	E
		Dyson, GW 369	Sorget For. Stn		0	3	S	35	33	E
		Gillet 16268	Nyeri	1900	0	25	S	36	57	E

		Kutilek, M 104	Nakuru	1767						
		Michelm 10895	Njoro	1981	0	20	S	35	57	E
		Moreau 3	Nanyuki	2103	0	1	N	37	4	E
		Napier, E.R 5794	Kedowa	1828	0	14	S	35	36	E
		N.I.B 35	Perkerra	1005	0	28	N	36	2	E
		Olson, D.24	Mutara ranch	1900						
		Owino, F 21	Nyandarua	2499						
		Oteke J, 65	Mt Kulal	2293	2	43	N	36	55	E
		Perdue & Kibuwa 9270	Nakuru	1767	0	56	S	36	35	E
		Reynolds, GW 6574	Nanyuki	1920	0	1	N	37	4	E
		Robertson SA 1614b	Gilgil, cultivated in Kirinyaga	2225						
		Robertson SA 1616c								
		Tweedie 2459	Thompson's Falls	2346	0	2	N	36	22	E
		unknown	Kekopey Ranch	1950						
		wabuyele & mabale 39	Nanyuki	1920	0	1	N	37	4	E
		wabuyele & Mbale 40	Gilgil	2100	0	32	S	36	19	E
		Wabuyele & Mbale 13	Hyrax Hill	1825	0	16.6	S	36	6.3	E
		Wabuyele & Mbale 16	Nanyuki	1920	0	1	N	37	4	E
		Wabuyele & Mbale 17	Nanyuki	1920	0	1	N	37	4	E
		Wabuyele & Mbale 41	Kagocho, Nanyuki	1920	0	1	N	37	4	E
		Wabuyele & Newton	Gilgil	2100	0	32	S	36	2.2	E
	A. lateritia var. lateritia	Glover Gwyne &Samwel 1253	Olkurto	2316	0	41	S	35	50	E
		Greenway, P.J 4080	Mayuni	365						
		Bally, P. R. O. 10686	Lumbwa	2133	0	12	S	35	28	E
		Bally, P. R. O. 11423	Mwanza	750	2	31	S	33	26	E
		Bigger, M. 2113	Lumi T2	1463						
		Bridgewood,S. , Abdalla, R.& Vollesen 1978	Mbagala	250	4	0	S	36	30	E
		Drummond and Hemsley 4289	Taita Hills	2050	3	25	S	38	20	E
		Faden *et al.* 20/293	Choke Hill	1640						
		Gilbert, V. C. 1191	Mt. Meru National Park	2103						
		Glover, Gwyne & Samwel 1338	Ol'pusimoru,	2590	0	49	S	35	50	E
		Glover, Gwyne & Samwel 1695	Ololung'a	2133	1	0	S	35	40	E
		Greenway 22034	Lushoto, cultivated in Nairobi		1	12	S	39	40	E
		Greenway, PJ 6448	Makuyuni	579	3	33	S	36	3	E
		Greenway PJ 10782	Musoma T1	1584						
		Harris, B. J & Harris, S. 4451	T6	70						

		Harris, B. J & Harris, S.1582	T6							
		Haveli 266/0	Kibaoni	400	8	8	S	36	41	E
		Minjas, A. N.& Raya, M. D. 1977		225						
		Nairobi Arboretum 3206	Taita Hills							
		Ngoundai 229	T3							
		Polhil, R & Paulo, S. 1443	Iringa	1500	7	46	S	35	42	E
		Leippert 5756	T2							
		Reekmans 10205	Burundi		3	0	S	0	38	E
		Reynods 6577	Voi		3	23	S	38	35	E
		Reynolds, G.W. 63690	Rombo	1524	3	15	S	37	40	E
		Vest-Frtzgerald, D. 7159	Arusha National Park		3	15	S	36	46	E
		Verdcourt, C 1755	Bomole Amani		5	6	S	38	37	E
		Wabuyele & mbale 27	Oloitokitok		2	53	S	37	35	E
SHWG	A. saponaria	Chriatian, H. B. 829	Inyanga	2103						
		Leach, L. B. 9390	Inyanga	2103						

Appendix 2: Specimens used in the study. All measurements are in centimetres

col. & no.	Ll	lw	mtn	ts	b	plo	pe	rs	rl	rd	sbl	sbw	snn	fbl	fbw	fnn	fdb	fdc	fda	fdf	lol	lof	stl	al	aw	ol	ow	sl
Mooney 9597	25		16	0.2	3	-	1.3	Subcapitate	11	7	1.8			1.5	0.4	5	0.7	0.3	0.7	0.5	3	0.5	1.7	0.3	0.1	0.6	0.1	
Gilbert 1150				0.1			1.9	subcapitate			2			1	0.2	8	0.5	0.4	0.6	0.4	2	0.35	0.8	0.4	0.1	-		
Gilbert, Ermias, Sebsebe,056863	16	4.3	6	0.2	3	8	1.8	capitate	5	7	1.2	0.7	11	1.4	0.5	9	0.6	0.4	0.6	0.4	3		2.4	0.4	0.1	0.7	0.2	2.4
Burger, W. 3615			8	0.2	2	8	2.4	capitate		6				1	0.4	1	0.5	0.4	0.4	0.5	3	0.4	2.2	0.3	0.4	0.5	0.1	1.9
Gilbert &Phillips 8862	41	9.7	8	0.2	5		1.3	cylindrical	27	7				1	0.3		0.5	0.3	0.6	0.5	3	0.4	2.1	0.4	0.1	0.7	0.2	2.4
Sebsebe, Fikre, Ermias Dagne, Afework 4238	9	2.5	13	0.1	2	16	2.5	capitate			1.8			1.5	0.3	6	0.5	0.2	0.4	0.5	3	0.8	2.8	0.35	0.1	0.7	0.2	2.8
Sebsebe 5528	26	6.5	10	0.3			1.9	cylindrical	16		2	0.5		1.7	0.2	6	0.5	0.3	0.4	0.4	3	0.8	2.3	0.4	0.2	0.6	0.1	2.3
Gilbert, Phillips with Damtew 9265U	20	4	15	0.2			3.5	Subcapitate	16	9				1.1	0.2	3	0.9	0.6	0.7	0.7	3	0.6	2.5	0.35	0.1	0.7	0.2	2.2
Sebsebe, Gilbert & Ermias 9304	16	4.2	6	0.2	3	11	1.7	capitate	6	8	2		14	1.3	0.4	5	0.6	0.4	0.4	0.6	3	0.5	2.1	0.5	0.2	0.6	0.2	1.8
Sebsebe 2945	14	4	8	0.1		14	1.9	capitate	6.5	8	2		8	1.2	0.2	3	0.4	0.2	0.3	0.3	3	0.6	2.5	0.35	0.2	0.7	0.2	2.4
Sebsebe 2426	13	5.5	6		7	17	2.7	Subcapitate	12					1	0.2	2					2	0.5	2.3	0.4	0.1	0.7	0.1	2.7
Ensermu, Muhammed, Tamene, 2520			8	0.3			2							0.9	0.2	3	0.5	0.3	0.6	0.6	3	0.75	2.3	0.45	0.2	0.7	0.2	2.2
Sebsebe 2558		6	6	0.1	7	22	1.9	capitate									0.5	0.3	0.4	0.4	3	0.8	2.3	0.4	0.1	0.7	0.2	2.4
Wabuyele	26	5.5	7	0.3	3	25	1.9	Capitate-subcapitate	9	10	3.5	1.5	16			3	0.9	0.4	0.5	0.4	4	1.3	2.9	0.35	0.1	0.8	0.2	3.2
Reynolds 7241						15	2	subcapitate	15	9				1.7	0.3	15	0.8	0.6	0.7	1.1	3	1	2	0.45	0.1	0.7	0.2	2
Latilo, MC	53	7.5	6	0.4		18	1.8	cylindrical	22	6	1.7			1.2		3	0.7	0.3	0.4	0.4	3	0.6	2.3			0.6	0.1	2
Kea					5	16	1.7	subcapitate	16	8	1.8	0.5	9	1	10		0.5	0.3	0.6	0.4	2	0.6	1.6	0.5	0.1	0.7	0.2	1.3
Hall, JB c495						12	1.3	cylindrical		5				0.8	0.8	3	0.6	0.2	0.3	0.3	3	0.8	2.3	0.3	0.1	0.6	0.1	2.3
unknown	26	6	8	0.5	3	15		cylindrical			1.7	0.9	7	1.2	0.3	3	0.4	0.2	0.2	0.4	2	0.5	2	0.3	0.2	0.6	0.2	1.7
Dyson, GW 369			5	0.5		14	2.5	capitate		?			5				0.5	0.2	0.4	0.4	4	0.7	3.1	0.60.3	0.1	0.6	0.2	2.3
Olson D.	12	3.5	10	0.4	3	13	1.9	capitate	5	8							0.4	0.2	0.4	0.4	3	0.65	2.2	0.3	0.2	0.7	0.1	2.6
N.I.B 35			5	0.8		?	2.5	subcapitate		?							0.4	0.2	0.4	0.4	3	0.7	2.1	0.25	0.2	0.7	0.2	2.3

unknown								capitate	10	11	5			3	0.2	7	0.6	0.2	0.4	0.4	3	0.6	2.7	0.35	0.1	0.8	0.2	2.1
Wabuyele & Newton	20	6.25	6	0.2	6	27	2	capitate		5	1.9	0.4					0.8	0.4	0.7	0.2	3	1	2.2	0.4	0.2	0.9	0.3	1.9
F. Owino 21	8	3	7	0.8		22	2.6	capitate	7	9	1.4	0.5		1.4	0.5	4	0.4	0.2	0.4	0.4	3	0.95				0.7	0.2	
Bally B13786	19	3.5	11	0.3	3	19	2.5	capitate	11	10							0.6	0.3	0.4	0.5	3	0.8	2			0.8	0.1	1.9
Brown E.S 1751								capitate	9	9							0.5	0.2	0.3	0.4	3	0.8	2.2	0.4	0.1	0.5	0.2	2.2
Lucas 273	26.6	7.16	7	0.4	3	28	2.7	capitate		10	3	0.8	13	1.4	0.3	3	0.5	0.3	0.4	0.4		0.8	2.5	0.3	0	0.8	0.8	2.5
Gillet 16268					6	17	2.1	capitate	5.8	9							0.5	0.2	0.4	0.2	3	0.6	2.6	0.3	0.1	0.8	0.3	2.6
M. Kutilek 104	13	3	6	0.4	4	15	2	capitate	6.7	8							0.5	0.2	0.4	0.5	3	0.6	2	0.25	0.1	0.6	0.2	2
Perdue &Kibuwa 8189	17.5	3.5	6	0.2	3	32	2.5	capitate	8	8				1.1	0.4		0.5	0.2	0.5	0.5	3	0.6	2.8	0.35	0.1	0.6	0.1	2.2
Perdue &Kibuwa 9270	31.5	6	10	0.1	5	26	2.5				2.3	1	13				0.5	0.3	0.5	0.2	3	0.9	2.3	0.3	0.1	0.6	0.1	2.6
Oteke J, 65	18.75	3.5	7	0.2	1		2.8	capitate	6	6	1	0.4		1			0.6	0.2	0.3	0.4		0.5cm	2.1	0.2	0.1	0.6	0.3	2
Guantai A.N.19	12	4	6	0.3		17	1.6	capitate	6.5	6	1						0.2	0.3	0.4	0.4		0.8	2.1	0.3	0.1	0.6	0.1	1.4
Bally PRO 8189	19	4	7	0.3		29	2.4	capitate-subcapitate	9	9			6	0.7	0.3	3	0.5	0.2	0.4	0.3	2	0.5	1.8	0.3	0.1	0.6	0.1	1.5
Michelm 10895	17.5	3	5	0.2	3	22	2.8	cylindrical	15	9	1	0.8					0.5	0.3	0.6	0.6	3	0.6	2.3	0.3		0.9	0.2	2.1
Robertson SA 1616c	21.5	3.5	5	0.3	4	30	1.8				1.5										3		1.6	0.4		0.6		1.6
Davidse 7041	17	7	6	0.4	2	36	2.6	capitate	8	8			3	0.9	0.2	3	0.4	0.3	0.6	0.3	3	0.5	2.2	0.5	0.2	0.7	0.1	1.8
PRO Bally 11424	30	6.3	8	0.1	5	22	1.5	capitate	5	7	1.4	0.3					0.4	0.3	0.6	0.6	2	0.4	2.2	0.4	0.2	0.4		1.6
Napier, E.R 5794	23	3.5	7	0.5		28	1.9	subcapitate	9	8			10	1.5		3	0.5	0.4	0.5	0.5	3	0.6	2.2	0.4	0.1	0.9	0.2	1.8
H.B Christian 10895	23	4.5	7	0.4		15	2	subcap-cylindrical			2	0.4		1.5		3	0.4	0.3	0.5	0.6	3	0.6	2.5	0.3	0.1	0.5	0.2	1.9
SA Robertson 1614b	27	5.5	5	0.4		38	3.6	cylindrical	16	13				1.5		3	0.6	0.4	0.6	0.5	3	0.6	2	0.4	0.1	0.6	0.1	1.9
Tweedie 2459		5.5	6	0.3		26	1.9	capitate	6	7	2	0.4	7	1.1		3	0.4	0.1	0.6	0.4	2	0.6	1.8	0.3	0.1	0.6	0.1	1.5
GW Reynolds 6574		5.5	5	0.3	3	18	1.9	capitate,conical	9	7			7	1.2	0.2	3	0.7	0.4	0.5	0.5	3	0.5	2	0.3	0.1	0.7	0.1	2.3
Moreau 3	18	5.5	6	0.3		29	1.5	capitate									0.5	0.2	0.3	0.4	3	0.7	2.5	0.3	0.1	0.8	0.1	2.3
Bally 13786	19	3.5	11	0.3	3	19	2.6	capitae	11	10			6				0.6	0.3	0.4	0.5	3	0.8	2	0.35	0.1	0.8	0.1	

Wabuyele & mbale 16	15	4	10	0.4	3	17	2	capitate	7	7	1	0.4	3	0.8	0.2	3	0.5	0.3	0.4	0.4	3	0.6	2.4	0.3	0.1	0.7	0.2	2.1
Wabuyele & Mbale 17	13	6	9	0.3	3	21		Capitate-subcapitate	15	7				1.3	0.2	7	0.6	0.3	0.3	0.3	3	0.6	2.5	0.4	0.1	1	0.2	2.3
wabuyele & Mbale 41	24	4.5	6	0.3	3	15	1.8	capitate	7	7				1.4	0.3	4	0.5	0.3	0.4	0.3	2	0.7	2.2	0.4	0.1	0.6	0.1	1.5
wabuiyele &Mbale 40	16	4.5	7	0.4		12	2	capitate-subcapitate	5	4			7	1.3	0.3	2	0.5	0.2	0.2	0.4	3	0.5	2.1	0.4	0.1	0.6	0.1	1.9
Wabuyele & Mbale 13	16	4.2	9	0.5	4	26	2.3	capitate	8.5	8	1.5	0.6	7	1.1	0.2	5	0.4	0.2	0.4	0.6	3	0.8	2.6	0.4	0.1	0.6	0.1	2.4
PRO Bally 10702	41	4	3	0.5	4	11	1.6	capitate	5	7	2.6	0.6					0.5	0.3	0.6	0.6	3		2.5	0.4				2.4
Glover Gwyne &Samwel 1253		5	6	0.4	5	18	2.3	capitate,	4.5	6	2	0.5				4	0.5	0.2	0.4	0.3	3	0.7	2.2	0.5	0.1	0.8	0.1	1.8
M.Bigger 2113	30		8	0.4	5	17	1.6	capitate		7	3	0.6	12	1	0.2	3	0.5	0.4	0.5	0.4	2	0.8	2.2	0.35	0.1	0.6	0.1	2.3
Greenway 22034	40	6.5	11	0.2	6	29	1.3	cylindical		7	5			1.3	0.2	2	0.6	0.5	0.7	0.4	2	0.5	1.4	0.4	0.2	0.6	0.1	
Greenway PJ 4080	45		6	0.3	5	14	1.2	subcapitae	6.5					1	0.4	2	0.6	0.3	0.5	0.4	2	0.5	1.8	0.25	0.1	0.8	0.4	1.8
PRO Bally B10686	33	7.5	9	0.3	3	26	2	capitate - cylindrical	9	8	3.5	1					0.6	0.4	0.5	0.5	3	0.9	2.2			0.6	0.2	2.1
Vest-Frtzgerald,D 7159			11	0.2	3	15	2.2	capitate	11	8	2.7	0.6					0.5	0.3	0.6	0.6	3	0.4	2.2	0.4		0.7	0.2	1.8
Greenway PJ 10782	27.5	4.5	5	0.2		17	1.9				1.6	0.6					0.5	0.3	0.4	0.5	2	0.4	2	0.35		0.5		2.2
R. Polhil & S. Paulo 1443			8	0.4	3	24	2.8				1.6	0.6		1.5							4		2.6	0.3		1.2		2.5
Leippert 5756	32	6	7	0.5	8	14	4.3	cylindrical			3.5	0.4					0.6	0.3	0.4	0.4	4	0.7	2.7			1	0.2	2.7
Harris Bj & Pocs 4451		3.45	7	0.7	3		2				3.2						0.6	0.2			3	0.6	3	0.4	0.1	0.6		2.2
Glover, Gwyne & Samwel 1338		5.1	9, 8	0.3		25	2.2	capitate		9																		
Bally PRO 11423			7	0.5		41	3.1	cylindrical	24	10	4.3	0.8					0.6	0.4	0.6	0.6	3	0.7	2.6	0.3		0.8		2.2
V.C Gilbert 1191	16.5	3.5	11	0.2		16	2.5	subcapitate			3	1	6	1		3	0.4	0.2	0.5	0.6	2	0.6	1.8	0.2	0.1	0.3	0.1	2
Gilbert VC 1191	38	3.5	11	0.2		16	2.5	subcapitate			3	1	6	1		3	0.4	0.2	0.5	0.6	2	0.6	1.8	0.2	0.1	0.3	0.1	2
Haveli 166/0	38	4.5	6	0.5	4	18	2.5	cylindrical	26	8	3	1	14	1.5			0.5	0.1	0.6	0.5	3	0.7	1.8	0.5	0.1	0.6	0.1	2.1
Bridgewood, Abdalla,Vollensen 1978			5	0.5		15	2.5	subcapitate-cylindrical	22	5	2	0.4	7	1.4			0.5	0.3	0.5	0.7	3	0.8	2.1	0.4	0.2	0.6	0.1	1.9
B. J. & S. Harris 1582						16	1.8							1.2	0.2		0.5				3		2.3	0.4		0.7		1.9
Reynold GW 6369	27	7	7	0.4			1.9	capitate	8	6				1		3	0.6	0.4	0.5	0.3	2	0.6	1.1	0.4	0.1	0.4	0.1	1.5
Verdcourt, C 1755	25	3.5	10	0.1		33	2.3	capitate	8	8	2.5	0.8	7	2		3	0.6	0.4	0.4	0.7	3	0.6	2.6	0.45	0.1	0.8	0.1	2

Bigger 2113	23	4.5	8	0.4	5	17	1.8	capitate	6	7	3.7	0.7	7	2		3	0.6	0.5	0.6	0.4	2	0.8	2.1	0.4	0.4	0.7	0.2	1.7
Greenway, PJ 6448	23	5.5	5	0.2	7	23	1.8	subcapitatel	9	7	6	1	7	1.7		3	0.6	0.4	0.6	0.4	3	0.6	2.2	0.3	0.1	0.6	0.1	2
Drummond and Hemsley 4289	34	7	7	0.2		16	3	subcapitate	12	9	4	0.8	7	0.9		3	0.6	0.3	0.4	0.3	3	0.6		0.2	0	0.9	0.2	2
Glover, Gwyne & Samwel 1695	20	5	7	0.3		26	2.8	capitate			2	1	7	0.8		3	0.5	0.3	0.4	0.4	3	0.9	2.5	0.5	0	0.8	0.1	3
Nairobi Arboretum 3206			7	0.2		25	3.4	subcapitate-cylindrical			4.5	0.8	9	2		3	0.4	0.2	0.3	0.3	2	0.7	2	0.3	0.1	0.8	0.2	1.6
Ngoundai 229	21	2.7	5	0.2	6	15	1.3	capitate	4	5	4.5	0.6	7	2		3	0.4	0.4	0.4	0.4	2	0.4	1.1	0.35	0.1	0.6	0.2	0.8

ll=Leaf length, **lw**= leaf width, **Mtn**=Marginal teeth per 5cm, **ts**=tooth size, **b**= No. of branches per inflorescence, **plo**=Peduncle length in flower, **Pe**= pedicel length in flower, **rs**=raceme shape, **rl**= raceme length, **rw**= raceme width, **sbl**= Sterile bracts length, **sbw**=sterile bracts width, **snn**, no. of nerve per sterile bract, **fbl**= Floral bracts length , **Fbw**= floral bracts width, **fdb**= floral diameter at base, **fdc**= floral diameter at constriction, **fda**= flora diameter above constriction, **fdf**= floral diameter at free/open end, **lol**= full length of lobe, **lof**= length of lobe free, **stl**= stamen length, **al**=Anther length, **aw**= Anther width, **ol**= Ovary length, **sl**= style length

Appendix 3: Species Descriptions after Reynolds (1966)

Aloe graminicola **Reynolds in *Journ. S. A Bot.* 19: 9 (1953)**

Plant succulent, solitary or forming small groups by division, acaulscent. Leaves about 16, densely rosulate, erectly spreading, lanceolate attenuate, up to 27 cm long, 7 cm at base, the apex dried and twisted; upper surface flat or slightly canaliculated, dull green with numerous dull white "H"-shaped spots, the spots irregularly scattered or sometimes more or less arranged in interrupted undulating transverse bans; lower surface paler green, with fewer spots, irregularly scattered or in transverse bands. Margins sinuate-dentate, with horny edge, armed with pale brown, pungent, deltoid teeth 4-5 mm long, 10-15 mm distant, the interspaces rounded and the colour of the leaf (sap dries yellow).

Inflorescence a branched panicle up to 1m high. Peduncle brown with grey powdery bloom, flattened and 2 cm broad low down, teret upwards, 3—5 branched from the middle or higher, the lowest branch subtended at the base by a rather fleshy many-nerved bract up to 3 cm long, 12 mm broad at base. Racemes capitate, densely flowered, the terminal averaging 5 cm long 8 cm diameter, the lateral a little smaller. Bracts narrowly deltoid acuminate, thin, scarious, 12 mm long, 2mm. Broad at base, 3—5- nerved. Pedicels averaging 20 mm. long.

Perianth scarlet to orange-scarlet (sometimes yellow), paler to yellowish at the mouth, averaging 33 mm. Long, slender with basal swelling 8mm. diam., constricted above the ovary to 5 mm. diam. Thence decurved, laterally compressed and enlarging towards the throat; outer segments free for 8mm., paler at the margins, the apices subacute, slightly spreading; inner segment broader than the outer, with broader white thin borders and more obtuse spreading apices. Filaments almost white, filiform-flattened, the three inner narrower and lengthening before the 3 outer, with their anthers in turn exerted 1-2 mm.; style pale yellow, filiform with the stigma at length exerted 2—3mm.; Ovary pale green 6 mm. Long, 2—5 mm diam. , finely 6-grooved; Capsule 32 mm. Long, 16 mm. diam. at the middle.

Aloe macrocarpa Todaro in *Hort. Bot. Panorm.* 1:36, t 9 (1875);

Plant succulent, aculescent or with very short stem, growing singly or suckering and forming small groups. Leaves 16—20, densely rosulate, suberectly spreading, lanceolate to lanceolate-

Inflorescence a branched panicle 1—1.25 m. tall. Peduncle basally plano-convex and 15—25 mm. broad, with 6—10 branches from above the middle, the lowest branch subtended at base by a thin subscarious ovate-acuminate, many-nerved bract 8—12 cm. and 15 mm broad. Racemes occasionally subcapitate and 8—12 cm. long but usually cylindric, slightly acuminate and 20—25 cm;ong, 8—9 cm. diam., the buds suberect, sometimes greenish-tipped, open flowers nutant to subpendulous. Bracts subscarious, narrowly deltoid, about 10 mm. long , 3 mm. broad, 3—5 - nerved, spreading to recurved at apex. Pedicels averging 20—25 cm long (sometimes 30mm.) long usually arcuate-ascending and nutant at apex.

Perianth glossy bright scarlet, orange-scarlet or coral-red, paler at mouth, often not glossy, 35—38 mm. long (occasionally 40 mm. long), basally flat and 1-— mm. diam. Across the ovary, abruptly constricted to 5—6 mm. above the ovary, thence enlarging to the throat and laterally compresses trigonously; outer segments free for 10 mm., paler at margins, 5-nerved, apices subacute, slightly spreading; inner segments broader than the outer and with more obtuse more spreading apices. Filaments lemon, filiform- flattened, the 3 inner narrower and lengthening before the 3 outer, with their anthers in turn exseted 1 mm.; stigma at length exserted 2mm.; ovary green, 8mm. long, 3 mm. diam.

Appendix 4: Number of teeth per 5cm of leaf length in the three taxa

	Taxon		
	A. macrocarpa	*A. lateritia* var. lateritia	*A. lateritia* var. *lateritia*
N	14	33	22
Mean	8.78	6.96	7.68
SD	3.44	1.92	2.07

n= no. of specimens mean= mean no. of teeth per 5 cm, SD= Standard deviation from the mean

Appendix 5: Variation in Style length

	Taxon		
	A. macrocarpa	*A. lateritia* var.graminicola	*A. lateritia* var. *lateritia*
n	15	35	21
Mean	2.26	1.96	2.1
SD	0.42	0.34	0.44