MORPHOMETRIC AND MOLECULAR COMPARISONS OF TWO ISOLATED POPULATIONS OF THE DESERT LOCUST, *SHISTOCERCA GREGARIA*, (ORTHOPTERA: ACRIDIDAE)

BY

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بسم الله الرحمن الرحيم

DECLARATION

I hereby declare that the work embodied in this thesis is a result of my own investigations during the three years research undertaken under supervision at the International Center of Insect Physiology and Ecology, Nairobi, Kenya, and has not been submitted before for any degree in any other Univeristy.

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DEDICATION

** This thesis is dedicated to my dear parents for love, prayers, encouragement and support throughout my whole life.*

** To my dear lovable wife, Eman for the sacrifies she made and the constant prayers she offered througout my study period.*

** To my children, Mohammed and Karim for the many hours they had to spend without their daddy around, which they never understood but bore so courageously*

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ABBREVIATIONS

A	Adenosine
ATP	Adenosine 5'-triphosphate
Amp	Ampicillin
bp	base pair
BCA	Bicinchoninic acid
BSA	Bovine serum albumin
C	Cytosine
conc	Concentration
CsCl	Caesium chloride
D^2	Mahalonobis distence
dATP	2'-deoxyadenosine 5'-triphosphate
ddATP	2',3'-dideoxyadenosine triphosphate
dCTP	2'-deoxycytidine 5'-triphosphate
ddCTP	2',3'-dideoxycytidine triphosphate
dGTP	2'-deoxyguanosine 5'-triphosphate
ddGTP	2',3'-dideoxyguanosine triphosphate
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleoside 5'-triphosphate
dTTP	2'-deoxythymidine 5'-triphosphate

ddTTP	2',3'-dideoxythymidine triphosphate
DTT	1,4-dithio-L-threitol
EDTA	Ethylenediaminetetra-acetic acid
EtBr	Ethidium bromide
G	Guanine
hr(s)	hour(s)
IPTG	Isopropyl-ß-D-thiogalactopyranoside
kb	kilobase pairs
kDa	Kilo-dalton
kg	Kilograme
km	kilometers
l	liter
LB	Luria-Bertani broth
min	minute(s)
M_r	Relative molecular mass
OD_{260}	Optical density at 260 nm
PAGE	Polyacrylamide gel electrophoresis
s	second(s)
SDS	Sodium dodecyl sulfate
T	Thymine
TBE	Tris-borate-EDTA

TEMED	N,N,N',N'-tetraethylethlenediamine
Tris	Tris (hydromethyl) aminomethane
U	units of enzyme activity
UV	ultraviolet (light)
v/v	volume/volume
w/v	weight/volume
X-Gal	5-bromo-4-chloro-3-indolyl-ß-D-galactopyranoside
a	alpha
ß	beta
m	micro

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بسم الله الرحمن الرحيم

"وما أوتيتم من العلم إلا قليلا"

صدق الله العظيم

SUMMARY

In Africa, there are two well known subspecies of the desert locust: the sahara subspecies, *Schistocerca gregaria gregaria* (Forskal) and the southern west subspecies, *Schistocerca gregaria flaviventris* (Burmeister). The sahara desert area, which is invaded by the *S. g. gregaria* is divided into three main regions: the north central region, south central and western regions. These populations are connected by migration circuits of swarms and gene flow occurs continuously between the populations. The invasion area of *S. g. flaviventris* covers an area of about 860,000 Km^2. It occurs in Namibia, South Africa, Botswana, Angola and Ascension Islands. It is known that the desert area of north Africa was in contact with that of southern Africa via an arid corridor across central Africa. Successive reduction in the aridity have isolated *S. g. flaviventris* population from *S. g. gregaria* when the corridor became filled with woodland and savanna unsuited to the locusts to breed. The objective of the present study was to evaluate the effect of the geographical barrier on the two desert locust populations, *Schistocerca g. gregaria* and *Schistocerca g. flaviventris*. The research focused on the degree of similarity or differences in: (a) the morphometrics by using the univariate and multivariate analyses;

(b) biochemical studies on the cuticular protein by using two dimensional gel electrophoresis technique and (c) genomic studies by using Random Amplified Polymorphic DNA (RAPD)-Polymerase Chain Reaction (PCR) technique. The findings are:

1. Morphometric comparisons by using univariate and multivariate discriminant analyses

Three different measurements (E, F, C) and two ratios (E/F, F/C), were selected for comparison of the morphometrics of the locust populations. The analysis of data was performed to examine the morphological relationships between the different populations with respect to their location only and to examine the relationships between the different forms (males and females) from the four different populations, with respect to their locations.

Univariate analysis technique using ANOVA test was performed for the four locust populations. The means of the different measured characters and the two ratios, indicate significant differences between the southern population and the other three northern populations. The mean values of E, F, C and for E/F ratio were lower for the southern population compared to the three northern populations. The coefficients of variances

(C.V.), were significantly different for all the measured characters. The highest C.V. was for the elytron character (80%) and the lowest was for the E/F ratio (33.41%). This indicates that, the four populations are highly variable in the length of the elytron, followed by those of the F and C measurements.

Multivariate analysis of variance using Wilk's Lambda and Hoteling-Lawley trace test, indicate that the four locust populations were significantly different ($P< 0.0001$). The first characteristic root, for the three measured characters (E, F, C), was 4.95 and the corresponding canonical variate explained 97.42% of the variability. This indicates that samples of both northern and southern populations differ considerably in a systematic way. The first characteristic root for the two ratios, was 5.07 and the corresponding canonical variate was 99.8%. The respective canonical correlations were highly significant ($P< 0.0001$).

Locusts representing different locations were shown in a canonical discriminant space for the three measured characters and for the two ratios. The southern population was represented in group 1 while the three northern populations were grouped together in group 2.

The Mahalanobis distances (D^2) between the centroids of the different populations for the three measured characters and for the two ratios were significantly different ($P< 0.0001$). The distances recorded between the southern and the northern

populations were higher than the distances between the three northern populations.

Standardized canonical coefficients for the three characters and for the two ratios are given. Weights for characters are listed for the first and the second canonical variate. The characters with the greater weights are more effective in population discrimination.

In the univariate analysis, means of the variable characters for different individuals representing the four populations were significantly different. The mean values of the southern males and females show that the former have smaller E, F, C values than the latter, but the means values for the E/F and F/C ratios were approximately the same. The coefficients of variances indicated significant differences between the different sexes for the three measured characters and the two ratios ($P< 0.0001$). The highest C.V. was for the length of the femur (40.18%) and the length of the elytron (38.49%), while the lowest C.V. was the E/F ratio (33.49%).

Multivariate analysis indicates that the two forms of the four population are significantly different from each other ($P< 0.0001$). The first characteristic root for the three measured characters, was 9.05 and the corresponding canonical variate accounted for 75.21% of the variability. The first characteristic root for the two ratios, was 5.16 and the

corresponding canonical variate accounted for 98.9% of the variability. The respective canonical correlations were highly significant ($P < 0.0001$).

Different sexes of the four different populations are shown in a canonical discriminant space for the three measured characters. There are differences in the morphology within the southern population. The females are grouped in group 1 while the males are grouped in group 2. The three northern populations (males and females) are grouped in a separate group 3. The canonical discriminant space for the two ratios, shows that the females and the males of the southern population have similar ratios (group 1) and they are separated from the northern population (group 2).

The Mahalanobis distance between the centroids of the males and females of the four populations showed that the separation between the southern males and females is smaller compared to those between the southern and the northern individuals. Thus the southern males and females are more morphologically closer to each other than to either sex of the northern locusts. Also, the two sexes of the northern populations are more or less similar in their morphological features.

2. Biochemical studies on the cuticular protein by using two dimensional gel electrophoresis technique

Differences in the protein composition of the cuticle of the two sexually mature locust populations (northern and southern), were demonstrated by the two-dimensional gel electrophoretic resolution of the abdominal protein. That of the northern, *S. g. gregaria*, males was resolved into six major bands, while that of the southern, *S. g. flaviventris*, males was resolved into three major bands only. Bands number 1, 2 and 3 were recorded in the two gels of the populations at the same molecular weight ranging from 21.5 to 31.0 kDa. On the other hand, bands number 4 and 5 at high molecular weights ranged from 70 to 97.4 kDa and band number 6 at low molecular weight (~ 20 kDa), were recorded only in the northern males. *S. g. gregaria*, females gave five major bands. These bands showed to be correlated with the bands from the northern males. Bands number 1, 2, 3, 4 and 5 were recognized in the two gels at the same molecular weights. However, band number 6 was recognized only in the northern males. Separation of the abdominal extracts of southern female locust, gave two major protein bands. Bands number 2 and 3 were recorded also in the northern females at the same molecular weights. Band number 1 was not recorded in the southern females as it shown in

the northern females. On the other hand, the protein profile of the southern females resembled that of the southern males except for the absence of low molecular weight (~ 25 kDa) protein (number 1) which is characteristic to the southern males only.

Thus, the major differences between the northern and southern abdominal protein compositions result from the presence or absence of bands number 4 and 5 corresponding to 70- 97.4 kDa, respectively.

3. Genomic analysis using Random Amplified Polymorphic DNA- PCR technique

Twenty primers were screened with the pool genomic DNA of both males and females of the two populations. Sixteen primers (A1, A3, A4, A5, A6, A7, A8, A9, A10, A11, A13, A14, A15, A16, A17 and A19), were examined and excluded from further screening because they produced homologous patterns as well as smeared or faint bands and over bright bands for any DNA templates.

Amplification product with four primers A2, A12, A18, A20, produced monomorphic and polymorphic patterns with the DNA pool samples. The fragments which differentiated the southern from northern locusts were scored at low molecular weights (100- 600 bp). However, three primers, A12, A18 and A20, failed to give

reproducible patterns with the different individuals and pools of the tested populations.

Primer A2, which produced diagnostic polymorphic patterns with the DNA pooled samples, was subjected to a second round of evaluation with individuals (5 males and 5 females) and pooled samples from the two sets of locusts. In the reaction, the amplified patterns were consistent and reproducible for any given DNA template. A distinct band (~ 600 base pairs), clearly visualized on Ethidium bromide agarose gel, was produced with template DNA derived from both males and females of the *S. g. flaviventris*. This band was not apparent in any of the RAPD-PCR products of templates derived from males and females of *S. g. gregaria*.

Southern blots of EcoRI digested genomic DNA of *S. g. gregaria* and *S. g. flaviventris*, were probed with the cloned 600 bp product of the southern males and females locusts. The results obtained from both hybridized membranes for individuals and pooled samples for males and females, were found to hybridize strongly to all males and females and pool DNA samples prepared from the southern locusts. This result revealed that sequences are highly repeated in the genome of the *flaviventris* population but not to the *gregaria* population.

The differences in the morphological, biochemical and genetic characters recognized between the south African locust population, *S. g. flaviventris*, and the northern population *S. g. gregaria* are due to the differences in the environmental factors and the genetic constitutions. The north African locust populations, *S. g. gregaria*, and the south African locust population *S. g. flaviventris* do not meet in nature, that is, the gene flow between them is prevented. This and our results suggests that the two populations have diverged into two different species. *S. g. flaviventris* represents an evolutionary dichotomy of the genus *Schistocerca* in the African continent.

Multivariate analysis of the morphometrics, two dimensional gel electrophoresis and RAPD-PCR techniques represent powerful tools in the differentiation of the locust populations, as in other insects.

Primers A2, A12, A18, A20 displayed specific amplified bands with the genomic DNA obtained from the southern locust *S. g. flaviventris*, which are not recognized by that from the northern *S. g. gregaria*. These primers are thus useful as genetic markers for differentiation between the northern and the southern locusts.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

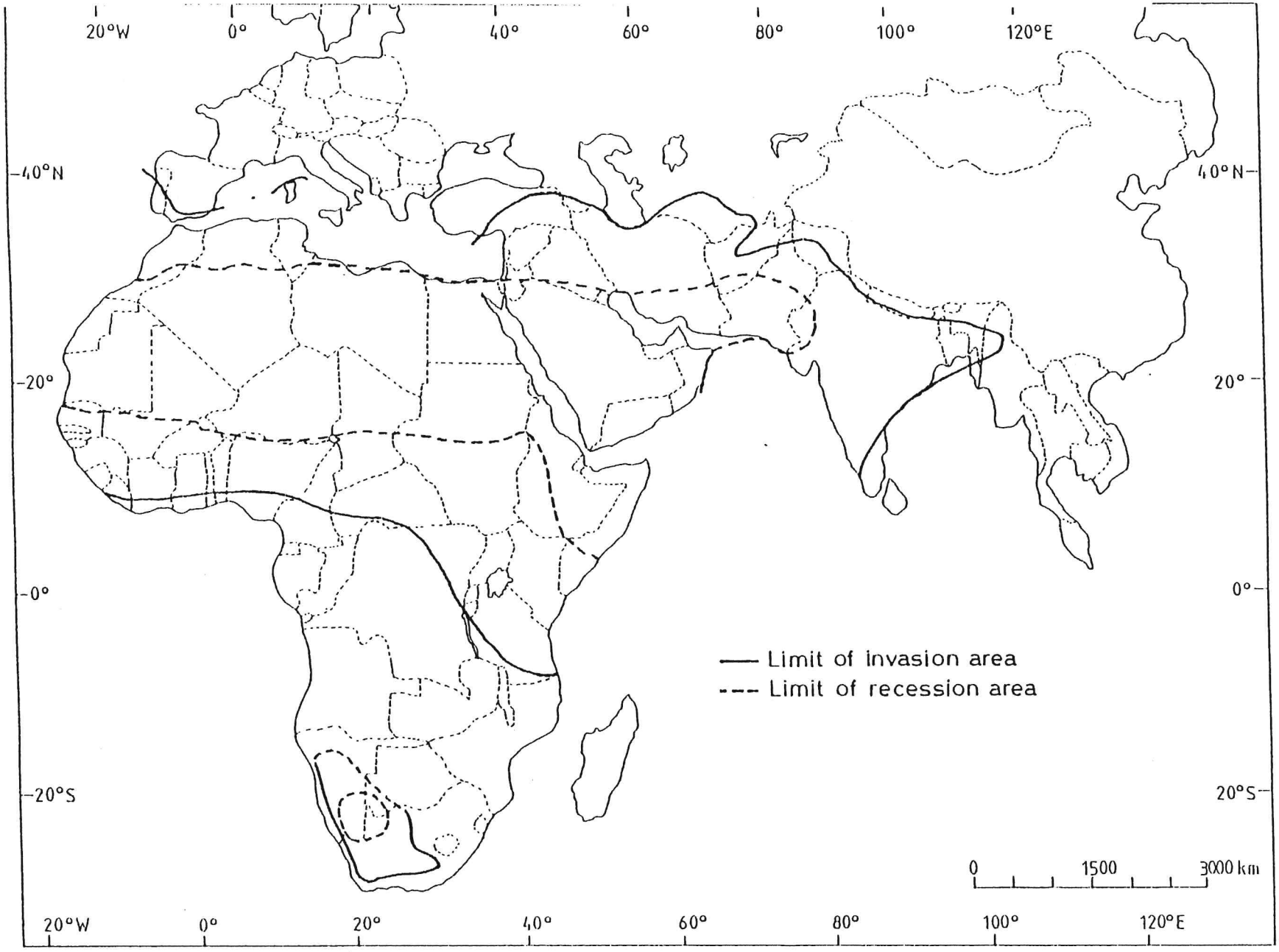
Since the biblical times it has been known that locust swarms cause great damage to grassland, agricultural crops and various other plants. These losses are not confined to Africa, India or Asia minor, which are the invasion territories of the desert locust, *Schistocerca gregaria*, but also affected other areas of the earth infested by other species of locusts. The desert locust is not a significant pest in its solitarious phase, in the recession areas, but becomes a major pest when gregarized and form swarms stimulated by the rain and improved vegetation (Nolte, 1974).

In Africa, the desert locust is one of four Acridid species of economic importance. The extent of its vast invasion area became appreciated only in the 1930 when Sir Boris Uvarov organized the systematic collection and analysis of its records and published a series of accounts of its 1926-1934 plague (Jago, 1970).

In Africa, there are two well known subspecies of the desert locust: the Sahara subspecies, *Schistocerca gregaria gregaria* (Forskal) and the southern west subspecies, *Schistocerca gregaria flaviventris* (Burmeister) (Meinzingen, 1993).

The Sahara desert area, which is invaded by the *S. g. gregaria* is divided into three main regions: the north central region, south central and western regions (Figure 1.1). The northern populations are connected by migration circuits of swarms or gene flow between the populations (Waloff, 1976). The invasion area of *S. g. flaviventris* covers an area of about 860,000 Km^2. It occurs in Namibia, South Africa, Botswana, Angola and Ascension Islands (Fig. 1.1). *S. g. flaviventris* is recognized as a subspecies separated from the north African populations in the degree of phase changes in the colouration (Nolte, 1965; Botha, 1967; Waloff, 1976; Harvey, 1981; Waloff and Pedgley, 1968). It is known that the desert area of north Africa was in contact with that of southern Africa via an arid corridor across central Africa. Successive reduction in the aridity could have isolated *S. g. flaviventris* population from *S. g. gregaria* when the corridor became filled with woodland and savanna unsuited to the locusts to breed (VanZinder-Bakker, 1976; Jago *et al.*, 1979)

Figure 1. 1 Invasion and recession area of the desert locust, *Schistocerca gregaria*, in the African continent



Subspecies in grasshoppers, in general, are usually a kind of averaging of the tiny inter-population differences with names attached only to what are presumably populations of insects carrying large numbers of integrated enzyme polymorphs, or a few polymorphs with dramatic externally observable features (Descamps, 1970). For future systematic work in the Acridoidea, it has been suggested that in addition to the criteria based on classical morphology, differences in internal organ morphology, genotype, karyotype, biochemical analysis should be taken into account (Jago, 1970).

Mayr (1963) pointed out that the use of trinomials does not always indicate the application of the modern species concept. Bolivar (1914) was one of the earliest to use trinomials, but leaves doubt the application of the trinomials, are applied to geographical races or to units previously defined by the term variety. The taxonomic problem is where to start drawing lines between geographical races, what nomenclature to use and how to indicate to outsiders, on what bases a certain taxa was raised. White (1968) mentioned that in groups where there are many cases of sibling species, subspecies and species complexes, it does not seem possible to carry out alpha-taxonomy (dealing with the description of species and arrangement in a comprehensive genera) in a fruitful manner and it would be more fruitful to proceed at

the beta level (dealing with species relationship) and the level of gamma-taxonomy (dealing with intra-specific variation).

The objective of the present study was to evaluate the effect of the geographical barrier on the two desert locust populations, *Schistocerca g. gregaria* and *Schistocerca g. flaviventris*. What has been the extent of the genetic divergence between the two populations? What is the degree of speciation between them ?

To answer the above questions, the research focused on :

1. The degree of similarity or differences in the morphometrics by using the univariate and multivariate analyses.
2. Biochemical studies on the cuticular protein by using two dimensional gel electrophoresis.
3. Genomic studies by using Random Amplified Polymorphic DNA (RAPD)-Polymerase Chain Reaction (PCR) technique.

1.2 LITERATURE REVIEW

1.2.1 MORPHOMETRIC COMPARISON USING UNIVARIATE AND MULTIVARIATE ANALYSES

The body size of an animal, of which morphometrics are a numerical expression, is dependent upon genetically transmitted characters and the modifying influence of the environment on them (Stower *et al.*, 1960). Morphometric methods are powerful research tools when used in the context of sound biological knowledge. Morphometrics allows one to summarize morphological data numerically and graphically, to express and test hypothetical relationships in many dimensions. There was a common opinion that the variance of size of organisms has a smaller genetic component than the variance of shape, hence more attention has been given to shape in systematic and evolutionary studies (Daly, 1985). On the other hand, Atchley (1983) has argued that size deserves more emphasis. Body size is heritable and is obviously a fundamental consideration in the ecogeography of an organism (Gould and Johanston, 1972). Many physiological processes are size related (Platt and Silvert, 1981). Studies on the geographic variation of *Apis mellifera* (Gadbin *et al.*, 1979), *Musca domestica* (Bryant, 1977), *Musca autumnalis* (Bryant and

Turner, 1978) and chromosomal races of morabine grasshoppers (Atchley and Hensleigh, 1974) indicate that size is the most important source of variation.

Qualitative characters are usually favored for describing and delimiting higher taxa of insects. The measurements of the wings of species in various genera and families of calyptrate Diptera, was found to be useful in assessing phenetic relationships. In this regard, canonical variate analysis has been used for ordination and cluster analysis of the pair-wise distance statistics for comparison between taxa. The results indicated that the wings of the flies are subject to strong stabilizing selection and exhibit moderately conservative changes among the taxa studied (Brown, 1979).

Plowright and Stephen (1973) chose 38 variables from the forewings of 60 species of the bee *Bombus* and 13 species of the closely related cuckoo bee *Psithyrus* in an effort to elucidate the generic relationships of the bumble bees and their parasites. Numerical taxonomic and canonical variate analyses yielded groupings of species of *Bombus* consistent with known subgenera and a single group for *Psithyrus*, thus supporting a monophyletic origin of the parasites.

The results of morphometrics analyses have been used by taxonomists to justify synonymy as well the recognition of new

taxa at the species level. Hogue (1982) examined 15 characters of male midges of the genus *Bibiocephala* in North America. Univariate and multivariate analyses indicated that no subsets clearly and consistently justified more than one of the four nominate species. Butler (1982) noticed two periods of adult emergence in a population of *Chironomus sp.* in Alaska. Univariate and bivariate analyses revealed two new species that were distinguishable as larvae, pupae, and male and female adults.

Distinct differences between proximate populations have been demonstrated by morphometric methods. Doyen and Slobodchikoff (1984) found size differences in microgeographic races of a coastal dune beetle within distances up to 500 m from the highest tide. Cornuet *et al.*, (1978) were able to distinguish between populations of honey bees in France by discriminant analysis of six characters. A partial discrimination was obtained between colonies of the same ecotype only 8 Km apart; complete discrimination was obtained between colonies of different ecotypes 120 Km apart.

Historical changes in present distributions of some insects have been reconstructed by morphometric comparisons. Foottit and Mackauer (1980) made a multivariate analysis of 21-33 characters of the European balsam woolly aphid that was introduced into

eastern North America several times before 1900. The divergence into three distinct populations was traced in the context of the aphid's parthenogenetic reproduction and colonization history.

Janson (1980) was able to reconstruct the history of postglacial distribution of the water boatman *Arctocorisa carinata* in northern Europe by a multivariate analysis of continuous and meristic characters. Isolated populations in previously glaciated areas could be recognized by their morphometric affinities to southern populations.

Kimani (1995) used the canonical discriminant analysis and the principle component analysis to separate the parasite *Cotesia flavipes* complex (Hymenoptera) into discrete groups. The canonical variate analysis indicated that there were three distinct groups in the *C. flavipes* species complex.

Morphometrics was first used in locust studies by Uvarov (1921). He employed pairs of measurements compounded into ratios to investigate differences between the supposed species *danica* and *migratoria* in the genus *locusta*. He showed that these were extreme polymorphs, which he termed phases, of the same species, the morphometric ratios providing a measure of the differences between the extremes of a continuous range of morphological variation.

In studies on the desert locust, *Schistocerca gregaria*, Maxwell-Darling (1934) gave a table of the morphometric data, including those for E: F (length of the elytron to length of the femur) ratios of seven populations showing different types of behaviour. Kennedy (1939) using the related morphometrics and behaviour of populations which he had observed, compared them with Maxwell-Darlings table and found marked divergences from the suggested associations. Rao (1942) used a number of ratios, including E: F, and divided their total ranges of values into three parts characteristic of the phases *solitaria, transiens* and *gregaria*, respectively.

Dirsh (1953) investigated a large number of body measurements and their ratios for adults of the desert locust, collected from a series of localities on different occasions, and referred to as being of phase *solitaria*, as well as for adults from swarms of phase *gregaria*. He concluded from a consideration of the means and ranges of different ratios that the best practical separation between the two extreme phases was given by the F: C (length of the femur to width of the head capsule) ratio. A sequel to the work of Dirsh (1953) was the graphical method proposed by Rungs (1954) taking into account both F: C and E: F ratios to differentiate between the phases of *S. gregaria*. Roonwal (1949) and Roonwal and Nag (1951) set up morphometric

limits for the phases *solitaria* and *gregaria* of the desert locust. They compared the measurements and the ratios of the characters E, F, C, P (length of pronotum at the keel), H (height of pronotum), and M (width of the pronotum at constriction), as well as sexual dimorphism and eye-strip number, made on samples taken from scattered populations in Mekran (Baluchistan) during 1935-6 and from swarms in India during the periods 1889-91 and 1930-1. There was no recorded evidence for the population histories before sampling. Misra *et al.*, (1952) using E, F, C and P, applied the method of discriminant function analysis to differentiate the phase status of various populations. The characters F, C, and P were all found to discriminate, but in varying degrees, C being the best. In the case of females, F also was a reliable discriminant. They pointed out that the sample of phase *gregaria* was possibly heterogeneous because the adults were collected from different localities in different seasons.

Albreeht and Blackith (1957) and Blackith (1957) used a modification of a method of analysis related to that employed by Misra *et al.*, (1952) in order to study phase status in *Nomadacris septemfasciata* (Serv.) and *Schistocerca gregaria* and also polymorphism in some Australian Acrididae. The form of multivariate analysis employed by Albreeht and Blackith (1957)

has distinct advantages over the previous single-character and ratio comparisons in that it makes much fuller use of all the morphometric data. It is more sensitive and can provide a concise geometrical representation of the relationships of morphometrics with various recorded environmental factors.

Stower *et al.*, (1960) used E, F, C measurements and the E: F and F:C ratios for 16 populations of the desert locust *S. gregaria*. They concluded that morphometrics may be used for the measurements of phase and phase change, only if the environmental factors under which particular populations developed are also taken into account.

Symmons (1968) described the derivation of a compound shape measure for discriminating between populations of the desert locust, *S. gregaria*. Three characters (E, F, C) were measured from twelve locust populations and multivariate discriminant analysis was conducted. He has argued that, the first canonical variate is a measure of phase. Deng *et al.*, (1996) used three parameters (E, F, C) and two ratios (E/F and F/C) for phase differentiation of the desert locust *S. gregaria*.

1.2.2 SEPARATION OF THE ABDOMINAL CUTICULAR PROTEINS BY TWO DIMENSIONAL POLYACRYLAMIDE GEL ELECTROPHORESIS

Insect cuticle is an extracellular layer, consisting mainly of proteins and chitin, which surrounds the whole insect. The cuticle functions both as skeleton and as a barrier towards the environment, and it is also responsible for the colouration of most insects, either by pigments in the cuticle or by submicroscopic structures (Neville, 1975; Hepburn, 1976). The ultrastructural organization of cuticle follows a general plan in all insect groups: long filaments of chitin are embedded in a matrix of protein, and most differences between cuticles are due to differences in the proteinaceous matrices and the way they are modified during maturation (Andersen *et al.*, 1986).

The cuticle consists of an outer thin epicuticle, containing lipids and proteins and often known to lack chitin, and a thicker procuticle, consisting mainly of chitin and protein (Silvert, 1985). The outermost part of the procuticle is deposited during the pharate period preceding ecdysis. Cuticular proteins deposited during this period are called either pharate proteins or pre-ecdysial proteins. The post-ecdysial proteins are formed in the inner part of the procuticle, the endocuticle. The

epicuticular proteins tend to be inextractable, and quantitatively they constitute a very minor part of the total cuticular proteins. The pre-ecdysial part of the procuticle is in some regions stabilized by the process of sclerotization, whereby the cuticle is hardened and the proteins are made inextractable. In other cuticular regions, the pre-ecdysial proteins remain unmodified, and the cuticle stays soft and flexible (Andersen *et al.*, 1995).

Proteins which can be extracted from pieces of cuticle may not be represented for the total mixture of proteins secreted, and the properties reported for proteins purified from various cuticles may not give a complete picture (Anderson and Barrett, 1971; Hackman, 1974).

Insect cuticles are often classified as either soft and plaint or hard and stiff materials. Much of the stiffness of cuticles is caused by sclerotization, a secondary modification of the cuticular proteins due to incorporation of oxidation products of catecholamine derivatives, but some of the stiffness is also inherent and apparently related to the pronounced hydrophobic character of the proteins in these cuticles (Hillerton and Vincent, 1993). The stiff proteins tend to have alkaline (high pH) isoelectric points, so they will be positively charged at physiological pH-values. The soft and pliable cuticles are

weakly sclerotized, and their proteins tend to be hydrophilic and to have acidic (low pH) isoelectric points (Vincent, 1975).

There is still some uncertainty with regard to where the cuticular proteins are synthesized, whether they originate somewhere else in the body and are taken up from the hemolymph by the epidermis and transported into the cuticle, or whether they synthesized in the epidermal cells. The close similarity between some of the proteins extracted from the hemolymph and the protein of the epidermal cells, has been shown by gel electrophoresis (Koeppe and Gilbert, 1973; Phillips and Loughton, 1976).

Cuticular proteins can be extracted with a number of solvents which affect different intermolecular types of chemical bonds. Solvents generally used for extraction are: distilled water; weak solutions of neutral salts, assumed to break electrostatic and van der Waals interactions between macromolecules; strong solutions of urea, assumed to break hydrogen bonds; and dilute sodium hydroxide, assumed to break stronger bonds, such as Schiff's bases and susceptible peptide bonds (Andersen, 1979).

Gel electrophoresis of proteins, is by far, the most widely used technique in insect molecular systematics. The technique relies on the fact that the identical proteins migrate the same distance under the electrical force applied to an electrophoretic gel, while nonidentical proteins usually migrate different

distances (Berlocher, 1984). Gel electrophoresis has proven useful at all three important systematic tasks: species discrimination, species identification, and hierarchical classification (Avise, 1974; Bush and Kitto, 1978). The amount of genetic variation within and between the natural populations is central to understanding the evolutionary processes. However, most of the early attempts to assess genetic variability were based on the analysis of segregation patterns in progeny of particular crosses, and they suffered from the inherent bias that only genes that exhibited variation could be recognized (Lewontin, 1974).

In the mid 1960s, one dimensional electrophoretic technique was applied to the problem of quantifying genetic variability and divergence in protein products of individual loci (Aquardo and Avise, 1981). This new technique could sample an array of proteins of about 20-50 polymorphic and monomorphic loci. The proteins assayed in conventional electrophoretic surveys may also be biased toward a more variable class of gene products (Leigh Brown and Langly, 1979).

Two dimensional gel electrophoresis (2-D), as introduced by O'Farrell in 1975, has allowed examination of a substantially increased number of protein-encoding loci. In 2-D technique, a mixture of denatured proteins is separated first on the basis of charge by isoelectric focusing, and then on the basis of

molecular weight by SDS slab gel electrophoresis. The technique is capable of separating as many as 1100 peptides from crude cell extracts on a single gel. The ability of this technique to resolve most protein variation resulting from charge-change is well documented as is the typical Mendelian behavior of the variants examined (Berlocher, 1984).

Two-dimensional techniques have been more widely used than one-dimensional gel electrophoresis to assay membrane and other structural proteins in addition to some water-soluble, largely enzymatic, proteins (Leigh Brown and Langley, 1979; Racine and Langley, 1980). Recent applications of 2-D to the analysis of intrapopulation variation have revealed substantially less genetic heterozygosity than had been estimated by single gel electrophoresis (SGE) (Liegh Brown and langley, 1979; McConkey *et al.*, 1979; Walton *et al.*, 1979; Racine and Langley, 1980). Aquadro and Avise (1981) used 2-D technique to compare an average of 189 polypeptides between six species of wild mice representing levels of evolutionary divergence ranging from different subspecies to different families. The magnitude of protein divergence estimated by 2-D was, on the average, only about one-half that predicted by SGE. This discrepancy may result from differences in sensitivities between the techniques or

differences in the mean level of variation and divergence between the sets of loci assayed by the two methods.

The level of heterozygosity in a wild population of the house mouse, *Mus musculus*, was estimated by means of 2-D gel electrophoresis for the whole kidney proteins. The observed level (2%) was substantially below the level detected by starch gel electrophoresis (Racine and Langley, 1980). This result was also similar to the results observed in the *Drosophila* populations (Leigh Brown and Langley, 1979).

The cuticular proteins of the migratory locust, *Locusta migratoria*, have been extracted from different parts of the body and analyzed by 2-D technique (Andersen *et al.*, 1986). An abrupt change in protein pattern is seen immediately after ecdysis, and the protein extracted from cuticle of pharate nymphs or newly emerged adults are different from the proteins extracted from the cuticle of mature nymphs or mature adults, respectively (Andersen and Hojrup, 1987). One- or two-dimensional gel electrophoresis of proteins extracted from abdominal cuticle of adult *Locusta* indicated that only quantitative changes occur in the protein pattern, and also pre-ecdysial cuticle dissected out two days before expected adult ecdysis gave the same protein pattern as cuticle obtained during ecdysis (Andersen *et al.*, 1995).

The pre-ecdysial (pharate) cuticle from adult locusts contain about 80% protein. About 8% of the protein cannot be extracted with 6 M urea. The inextractable fraction is presumably covalently linked to the chitin fraction. The major part of the proteins can be extracted with acidic (<pH 4) or alkaline (>pH 10) solutions, whereas only little protein is extracted at pH-values between 7 and 10 at 20° C (Andersen *et al.*, 1986).

1.2.3 RANDOM AMPLIFIED POLYMORPHIC DNA-POLYMERASE CHAIN REACTION (RAPD-PCR)

Molecular systematics encompasses three main areas of studies: population structure studies (geographic variation, mating systems, heterozygosity, and individual relatedness), identification of species boundaries (including hybridization) and estimation of phylogenies. The genetic structure of a population is perhaps the most fundamental piece of information for a species that requires management (Hills and Mortiz, 1990).

In the past few years there has been a remarkable increase in the application of the DNA analysis to problems in population genetics and systematics. Population genetics deals with genetic changes that occur within populations and evolution is a process of change in the genetic makeup of populations (Li and Graur, 1991). Analysis of DNA has several significant advantages; the genotype rather than the phenotype is assayed, one or more sequence appropriate to inheritance can be made; DNA can be prepared from small amounts of tissue, is relatively stable and the structure of DNA being simple linear sequences of only four nucleotide bases (Post *et al.*, 1992).

There are several approaches of assaying DNA variation in the molecular genetics. Restriction Fragment Length Polymorphism

(RFLP) has become widely used for the construction of genetic maps of many species and for mapping of genetic traits (Ganal *et al.*, 1990; Tanksley *et al.*, 1989). The assay is usually practiced with species-specific probes; it requires DNA blotting and hybridization of several micrograms of DNA, which has been digested with a restriction endonuclease. Radioactive probes are usually used with each probe detecting one or more genetic loci that share sequence homology. Each allele at a locus is identified as a mobility variant of an endonuclease restriction fragment. The RFLP requires well equipped laboratories and is relatively laborious (Rafalski *et al.*, 1991).

Recently, two groups independently and simultaneously described a novel polymorphism assay. Williams *et al.*, (1990) described genetic mapping application and called the new method the RAPD assay, for Random Amplified Polymorphic DNA. Another group, Welsh and McClelland (1990) focused on genome fingerprinting and called their assay Arbitrarily- Primed PCR (AP-PCR). Both of these assays are based upon the observation that a single, short oligodeoxynucleotide (10 bp) of randomly chosen DNA sequence, with at least 50% GC (Ganidine-Cystine) content are preferred. Only 15-25 nanograms of genomic DNA templates are needed. The amplification is preformed in the presence of dNTPs as substrate and catalyse by *Taq* polymerase

enzyme. In order for amplification to occur, low annealing temperature allow the primers to anneal to the arbitrary regions of the genome which are complementary to the primers. The primers annealing sites must be separated at a distance of no greater than 3000 bp as this is the maximum size that can be amplified with routine PCR. The observation that single substitutions, especially in the 3' end of the primer, can change amplified banding pattern, implies that annealing in RAPD-PCR must be precise (Williams *et al.*, 1991a).

The reaction products are separated on standard agarose gels and visualized with Ehidium bromide staining. The nature of the fragments that are amplified, is highly dependent on the primer and the genome sequences. DNA amplification with random sequence primers is a highly sensitive method for discovering polymorphism randomly distributed throughout the genome. A primer usually amplifies several bands, each originating from a different genomic location. The multiplex nature of the RAPD assay results in increased analytical throughput (Black, 1993).

Absence of a fragment presumably occurs because amplification cannot proceed on DNA strands from either of the homologous chromosomes in an individual. This can occur through point mutation at one or both primer annealing sites on a DNA strand, inversions surrounding a site or insertion that separate

the annealing sites at a greater distance than can be amplified (Rafalski *et al.*,1991).

Arbitrary primed PCR is not quantitative and consequently it is unknown whether individuals whose DNA yields a specific fragments are heterozygous (have one copy) or homozygous (two copies) for an amplifiable allele. Alleles at arbitrary primed loci therefore segregate as dominant markers (Williams *et al.*, 1990).

Williams *et al.*, (1991b) reported that more than 95% of the loci amplified in *Nurospora crassa* contained dominant alleles. Hunt and Page (1992) reported that 90% of alleles segregated as dominant markers in *Apis mallifera*. Black *et al.*,(1992) showed that RAPD-PCR could be used to identify polymorphism within and among greenbug (*Schizaphis graminum*) biotypes, an aphid species for rare allelic variation between biotypes was detected at only a single allozyme locus. Abid *et al.*, (1989) have found variation among *Acyrthosiphon pisum* and *Uroleucon ambrosiae* individuals from single plants and demonstrated polymorphism within two endoparasitic wasps, *Lysiphlebus testaceipes* and *Diaretiella rapae*.

Puterka *et al.*, (1993) used allozyme and RAPD-PCR markers to examine genetic relationships among populations of the Russian wheat aphid (*Diuraphis noxia*). Three of twenty allozyme loci

were polymorphic and cluster analysis with these markers was only able to separate thirty-five populations into five groups. In contrast, cluster analysis with RAPD-PCR polymorphism placed populations into the same five groups but further separated populations within each group. A large number of polymorphism has been detected in honey bees with RAPD-PCR (Hunt and Page, 1992).

Roehrdanz *et al.*, (1993) found intraspecific variation in two endoparasitic hymenoptera species and extensive intrspecific variation among closely related species. Roehrdanz and Flanders (1993) also found interaspecific variation in four coccinelid species. Perring *et al.*, (1993) were able to use arbitrary primers to identify variation within and among whitefly taxa in the genus *Bemesia*.

Chapco *et al.*, (1992) used RAPD-PCR markers to identify intraspecific variation within *Melanoplinae* and *Oedipodinae* (grasshopper) species. RAPD-PCR distinguished subspecies in *Aedes aegypti* (Ballinger-Crabtree *et al.*, 1992). Kambhampati *et al.*, (1993) used RAPD-PCR loci as species diagnostic markers in *Aedes scutellaris* group. Wilkerson *et al.*, (1993) used RAPD-PCR to distinguish the two cryptic species *Anopheles gambiae* and *An. arabiensis*. Apostol *et al.*, (1993) described a technique to use

RAPD alleles as fingerprinting markers in estimating the number of full sibling families at an oviposition site.

Hymer *et al.*, (1993) used RAPD-PCR to distinguish different populations of the Mediterranean fruit fly, *Ceratitis capitata*.

The populations used include strains which differ primarily in terms of geographical origin. In addition, strains that have been in a long term laboratory culture can also be distinguished from wild caught strains. Kimani (1995) used RAPD-PCR to differentiate the *Cotesia flavips* complex (Hymenoptra).

The advantages of these techniques (RAPD-PCR, AR-PCR) are that they do not require cloning or DNA sequence information for primer design, they do not employ labelling with radioactive markers, and genetic polymorphism can be visualized within 24 h from extraction of genomic DNA. This contrasts with techniques that employ Southern analysis (RFLP) in which the researcher must wait a minimum of 5-7 days to visualize polymorphism. The large number of bands amplified in individuals, permits their use in genetic fingerprinting, species diagnostics and differentiation of cryptic species (Black, 1993).

CHAPTER TWO

MORPHOMETRIC COMPARISONS USING UNIVARIATE AND MULTIVARIATE DISCRIMINANT ANALYSES

2.1 Introduction

Analysis of geographical variation within species continues to receive attention by taxonomists. Character differences between populations have traditionally been compared using univariate statistical procedures. Increasingly, more multivariate statistical studies have been used to examine geographical variations in insect populations (Gould and Johnston, 1972; Niles, 1973; Kamani, 1995).

The use of multivariate analysis in systematics has been formalized in several ways. It includes both canonical discriminant analysis and generalized distance, or the Mahalanobis distance (D^2). The discriminant analysis model, also known as the classification criterion, is based either on the individual within-group covariance matrices or the pooled covariance matrices (Zimmerman and Ludwing, 1974; Fargo *et al.*, 1986).

Canonical discriminant analysis is a dimension-reduction technique related to the principal component analysis and canonical correlation. Given two or more groups of observations, with measurements on several quantitative variables, canonical discriminant analysis derives the linear combination of the variables with the highest possible multiple correlation with the groups (Inayatullah *et al.*, 1987). The maximal multiple correlation is designated as the first canonical correlation. Canonical correlation is a generalization of correlation and regression that is applicable when the attributes of a single group of objects can be divided naturally into two sets. Canonical correlation calculates overall correlations between two sets of variables. Linear combinations within the first set of variables and within the second set are considered simultaneously and the linear combinations that maximize the correlation between the two sets are selected. The coefficients of the linear combinations are designated as the canonical coefficients or canonical weights. The variable defined by the linear combination is the first canonical variable or canonical component. The second canonical correlation is obtained by finding a linear combination uncorrelated with the first canonical variable, but having the highest multiple correlation with the groups (SAS Institute, 1988).

The relationship between the groups is measured by the Mahalanobis distances between their centroids (Mahalanobis, 1936; Rao, 1952). Small distances indicate close relationships between groups, whereas greater distance indicates more distant associations. Distance analysis determines how physiology, anatomy, behaviour, or ecology of one population contrasts with those of other populations (Pimentel, 1979).

The objective of this chapter was to use univariate and multivariate discriminant techniques to evaluate the differences between the northern and the southern African locust populations, and to quantify the extent of variation present between these populations.

2.2 Materials and Methods

2.2.1 Insects

Four different locust populations from different localities, were selected for the study. The north African gregarious populations, *Schistocerca g. gregaria*, were freshly collected from ICIPE field station at the Red Sea in the Sudan (f), and from Ethiopia (Ethiopian field population); (e), and the third was the Red Sea population (r), reared at the ICIPE insectary, Nairobi, which has been reared for twenty generations. The South African gregarious population (s), *Schistocerca gregaria flaviventris*, was collected by Dr. Dick Brown (Plant protection institute, Protoria) from the Kalahari desert in the Republic of South Africa. The males and the females of the different populations, were collected from the field as fledglings.

Insects were reared in the Insect and Animal Breading Unit (IABU) of ICIPE, according to the method described by Ochieng-Odero *et al.*,(1991). Gregarious locusts were maintained under crowded conditions (~ 200 per cage), the four locust populations were kept at 28-34^0 C, 40-50% relative humidity and daily illumination of 12 hours. The diet consisted mainly of fresh sorghum plants and wheat brans. After two weeks from maturation,

twenty males and twenty females from the different populations were collected and subjected to morphometric measurements.

2.2.2 Choice of the characters and the measurements

Three characters were selected, the length of fore-wing (E), posterior femur (F) and the widest part of the head capsule (C) (Deng *et al.* 1996). The characters were measured using an electronic caliper (Trimos Sylvac Metrology Ltd, London, UK, range of 0-150 mm, accuracy $\pm$ 0.03 mm), according to the method described by Ochieng- Odero *et al.*, (1991). Two ratios (E/F, F/C) were also calculated and compared for the different populations.

2.2.3 Data analysis

The measurements were analyzed using One-way Analysis of variance (ANOVA) and Multivariate discriminant analysis of variance. The data analysis were performed using the Statistical Analysis System software (SAS version 6.04, SAS Institute, 1988).

2.3 Results

Three different measurements (E, F, C) (Fig. 2.1) and two ratios (E/F, F/C), were selected for comparison of the morphometrics of the locust populations. The analysis of data was performed to examine the morphological relationships between the different populations with respect to their location only and to examine the relationships between the different forms (males and females) from the four different populations, with respect to their locations (Appendix A).

A. Comparison with respect to location

Univariate analysis technique using ANOVA test was performed. The means of the different measured characters and the two ratios indicated significant differences between the southern population and the other three northern populations (Table 2.1). The means values of E, F, C and for E/F ratio (except F/C ratio), were lower for the southern population compared to the three northern populations.

The coefficients of variances (C.V.) were significantly different for all the measured characters (Table 2.2). The highest C.V. was for the elytron character (80%) and the lowest

Figure 2.1 Diagrammatic scheme of the three measurements of the desert locust, *Schistocerca gregaria*. E: length of elytron; F: length of femur; C: width of head capsule.

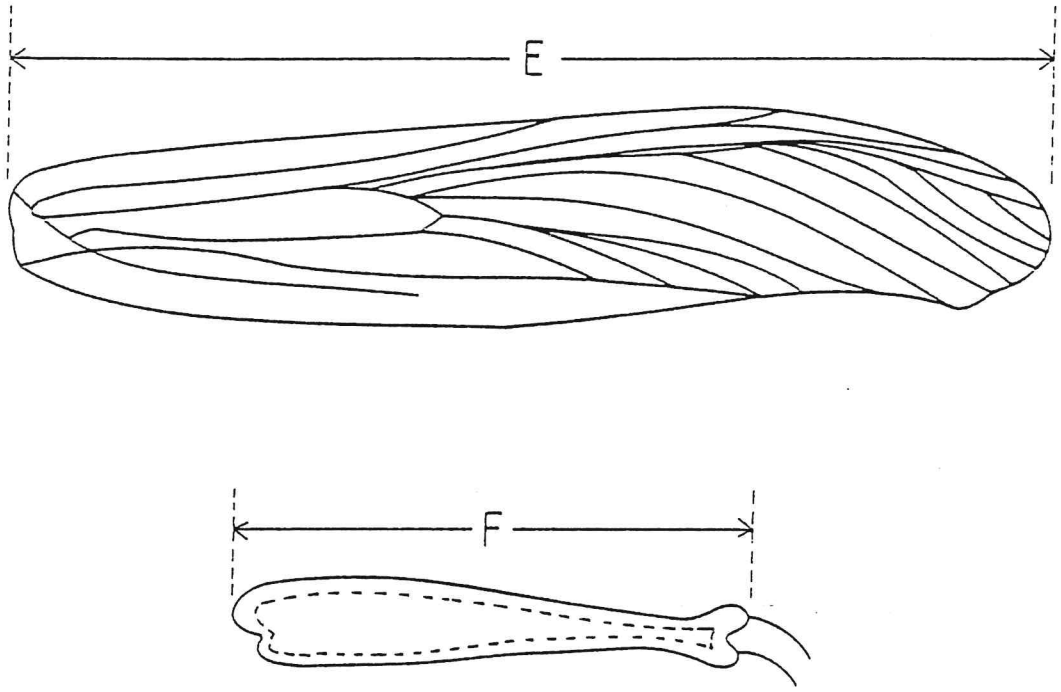


Table 2.1 Mean values and standard errors of different morphological characters for the four locust populations, *Schistocerca gregaria*

POPULATION/CHARACTER	E	F	C	E/F	F/C
f	58.03 ±0.79a	26.55 ±0.32a	7.40 ±0.09a	2.18 ±0.01b	3.58 ±0.02b
r	57.57 ±0.81a	26.20 ±0.35a	7.34 ±0.09a b	2.19 ±0.01b	3.57 ±0.02b
e	55.17 ±0.58b	26.02 ±0.27a	7.12 ±0.07b	2.24 ±0.01a	3.45 ±0.02c
s	52.87 ±0.70c	24.64 ±0.37b	6.24 ±0.08c	2.03 ±0.01c	4.16 ±0.02a

* Means followed by the same letter in a column are not significantly different (P = 0.001).

* E: elytron length F: femur length C: head capsule width.

* f: red sea population r: Ex-Addis population

* e: Ethiopian population s: Southern population.

Table 2.2 Coefficient of variations in characters of the desert locust populations, *Schistocerca gregaria*

VARIABLES	C.V. %	Pr > F
E	80.00%	0.001
F	78.80%	0.003
C	71.50%	0.001
E/F	33.41%	0.001
F/C	35.40%	0.001

* E: elytron length F: femur length C: head capsule width

was for the E/F ratio (33.41%). This indicated that, the four populations were highly variable in the length of the elytron, followed by variation in the F and C values.

Multivariate analysis of variance using Wilk's Lambda and Hoteling-Lawley trace test (Lindeman *et al.*, 1980) indicated that the four locust populations were significantly different ($P< 0.0001$). Discriminant analysis grouped the similar morphometric locusts in one group and the different morphometric locusts into other groups. In order to account for the relationships between the variables and to obtain a three dimensional representation, the E, F, C axes are inclined at angles depending on the correlations between them, and the sum of the squares of the distances between sample means is then D^2. The sample means also will form a cloud of points in three dimensions and in order to reduce the dimensions, the first canonical variate is calculated. The first canonical variate is thus the best single linear compound measure, based on values of E, F, C, E/F and F/C. The first characteristic root for the three measured characters (E, F, C) was 4.95 and the corresponding canonical variate explained 97.42% of the variability (Table 2.3). This indicates that samples of both northern and southern populations differed considerably in a systematic way. The second characteristic root was 0.13 with a

corresponding canonical variate that explained 2.57% of the variability. The first characteristic root for the two ratios was 5.07 and the corresponding canonical variate was 99.8% , while the second characteristic root was 0.01 and the corresponding canonical variate explained 0.2% of the variability (Table 2.4). The respective canonical correlations were highly significant ($P< 0.0001$).

Locusts representing different locations were shown in a canonical discriminant space for the three measured characters (Fig. 2.2) and for the two ratios in Fig. 2.3. Two different and well separated groups were obtained, are representing the northern populations (group 2) and the southern population (group 1).

The Mahalanobis distances (D^2) between the centroids of the different populations for the three measured characters are shown in Fig. 2.4 and for the two ratios in Fig. 2.5. The distances between the four different populations were significantly different ($P< 0.0001$). However, the distances between the southern and the northern populations were significantly higher than those between the three northern populations.

Standardized canonical coefficients for the three characters and for the two ratios are given in Table 2.5. Weights for characters were listed for the first and the second canonical variate. The characters with the greater weights are the most

Table 2.3 Characteristic roots and the propotions of the three measured characters for the four desert locust populations, *Schistocerca gregaria*

First characteristic root (Eigenvalue)	Proportion (%)
4.95	97.42
0.13	2.57

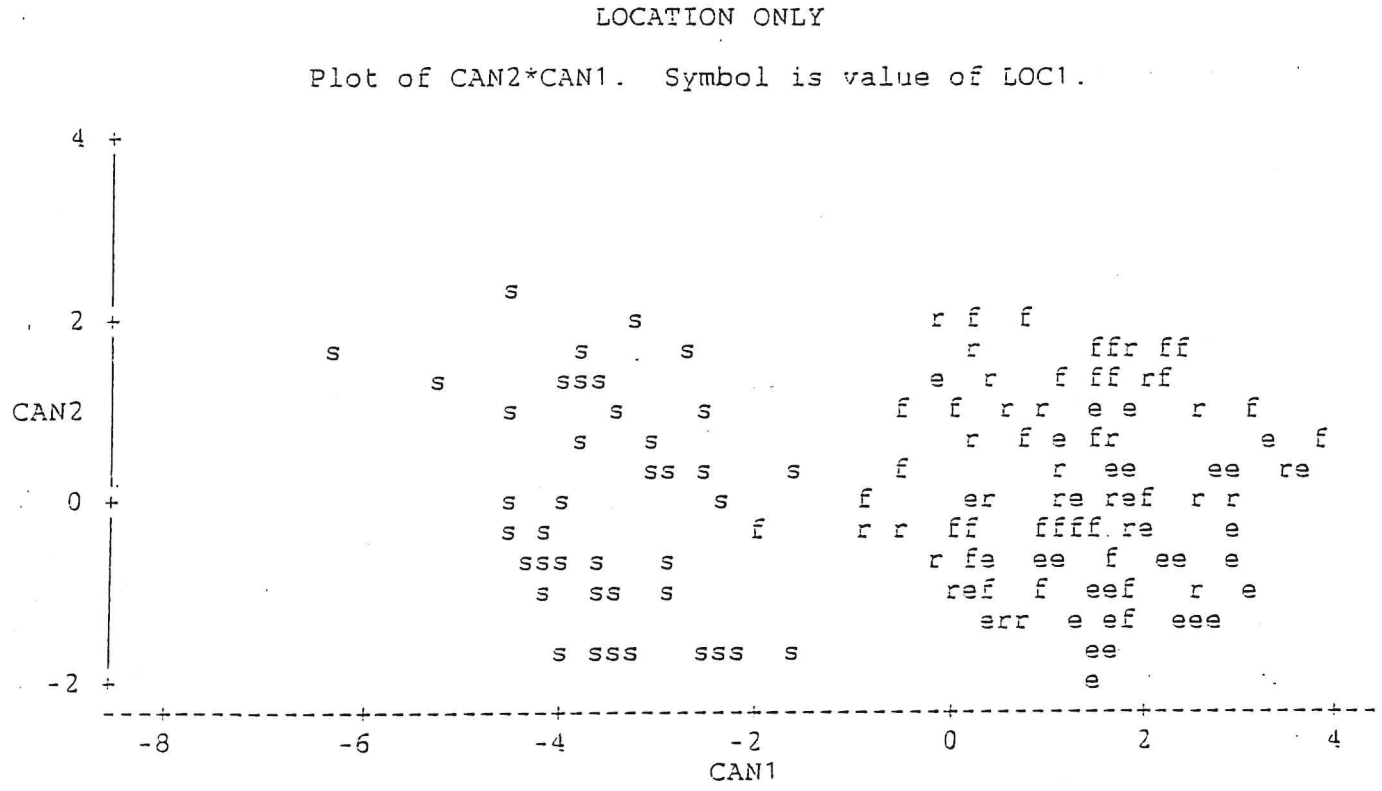
Table 2.4 Characteristic root and the proportions of the two ratios for the four different locust populations, *Schistocerca gregaria*

First characteristic root (Eigenvalue)	Propotion (%)
5.07	99.80
0.01	0.20

Table 2.5 Standardized canonical coefficient of the measured characters and ratios for the four desert locust populations, *Schistocerca gregaria*.

Characters	Can 1	Can 2
E	0.44	- 0.59
F	- 2.32	1.22
C	2.79	0.48
E/F	0.26	1.58
F/C	- 2.22	1.30

Figure 2.2 Plot representing the four different desert locust population along the two axes of canonical discriminant space for the three measured characters (E, F, C). f= Red sea field population; r= Red sea lab. population; e= Ethiopian population and s= Kalahari field population. Group 2= north African desert locust populations and Group 1= south African desert locust population.



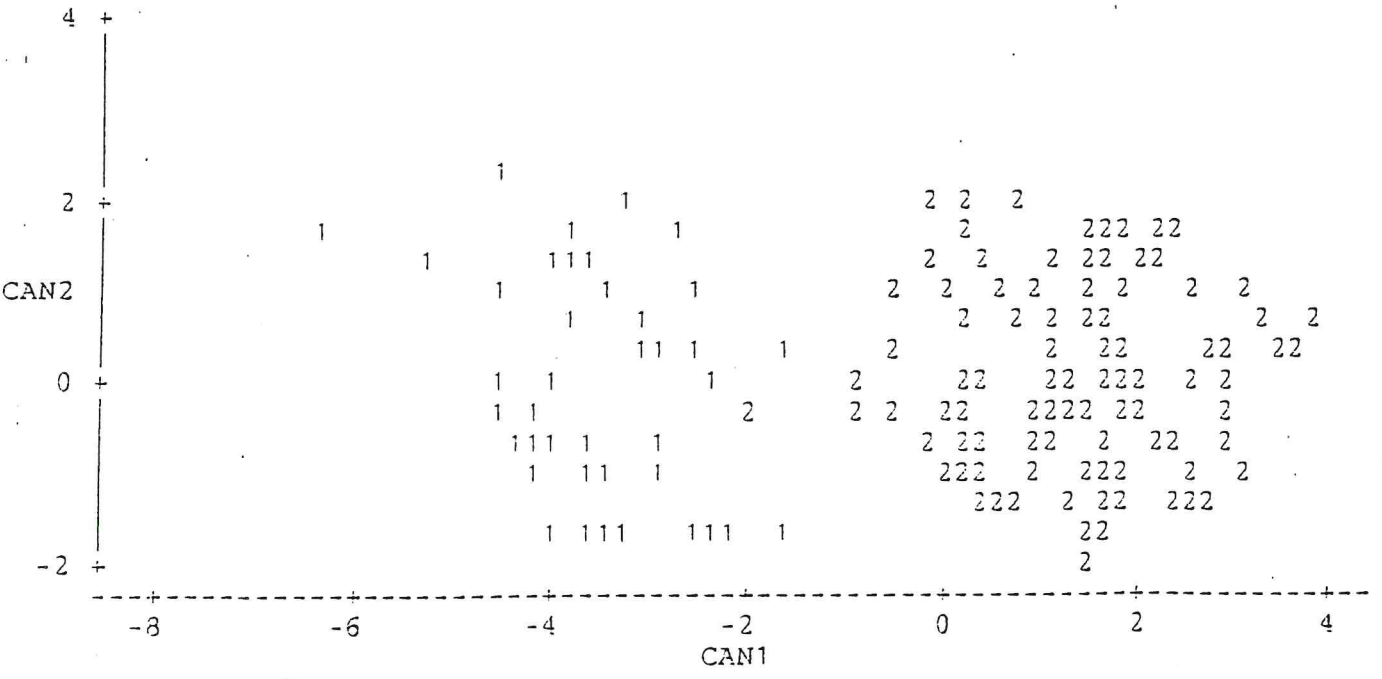
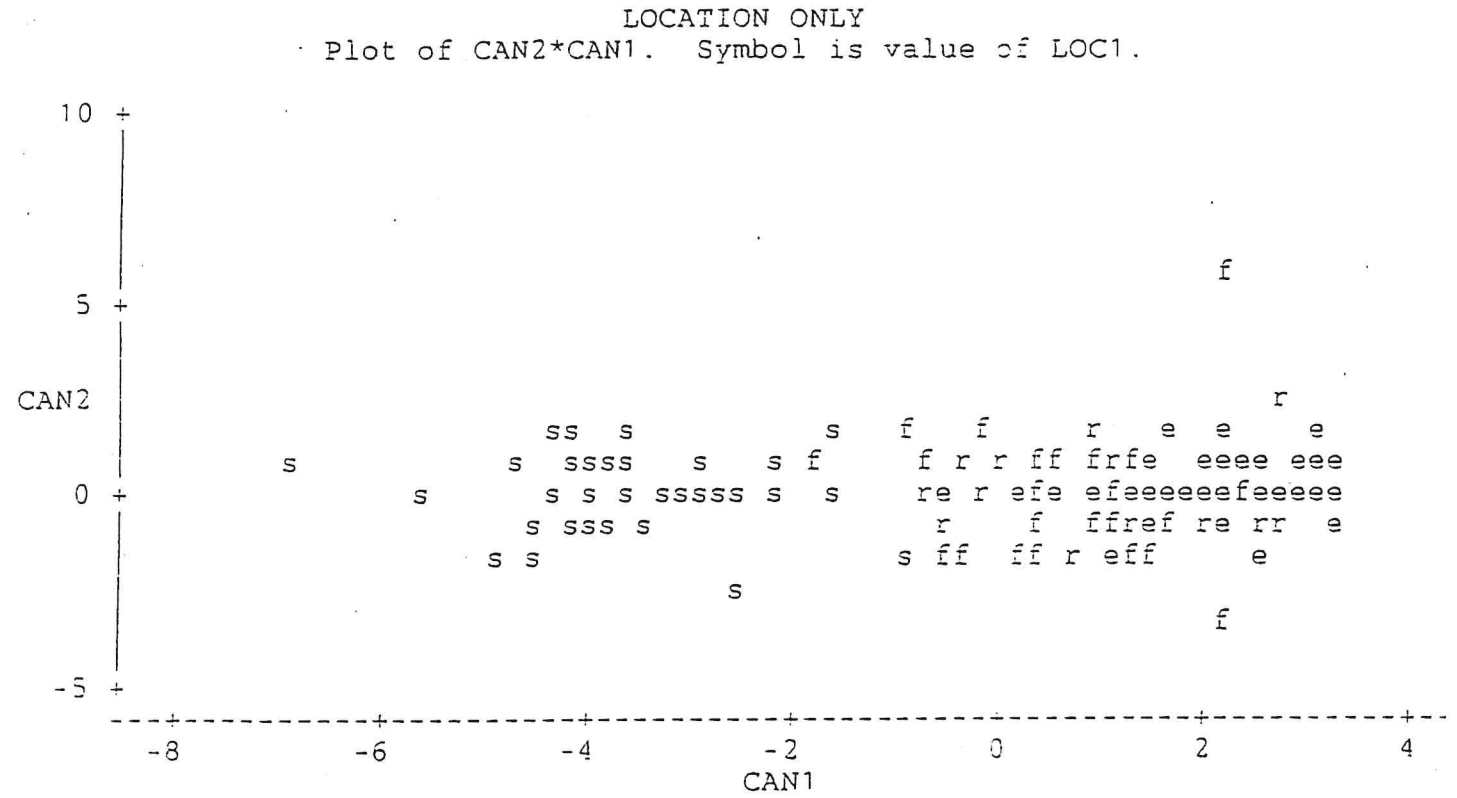


Figure 2.3 Plot representing the four different desert locust population along the two axes of canonical discriminant space for the two ratios (E/F, F/C). f= Red sea field population; r= Red sea lab. population; e= Ethiopian population and s= Kalahari field population. Group 2= north African desert locust populations and Group 1= south African desert locust population.



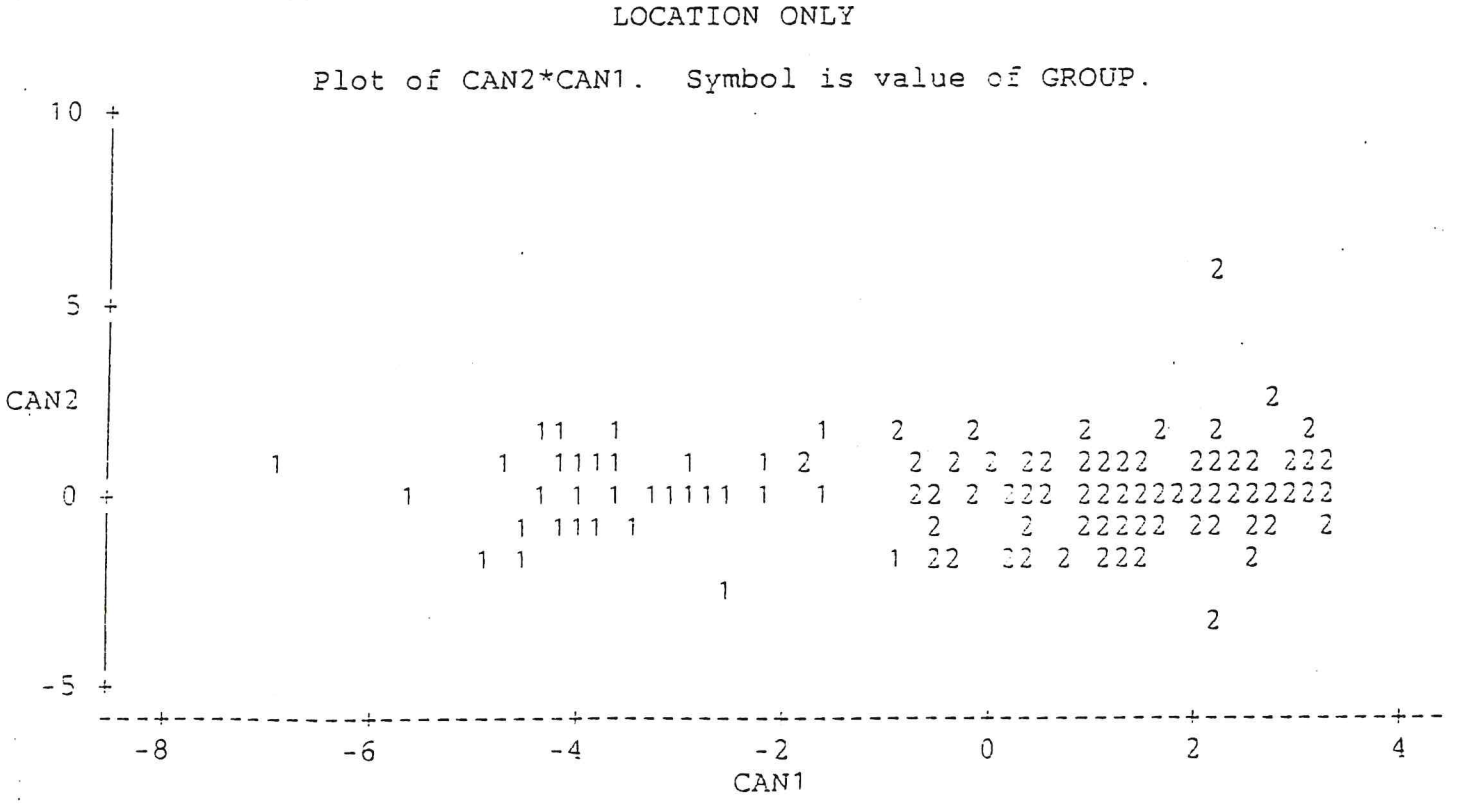


Figure 2.4 Mahalonobis distance relationships for the three measured characters (E, F, C) among the four different locust populations of, *Schistocerca gregaria*. f= Red sea field population; r= Red sea lab. population; e= Ethiopian population and s= Kalahari field population

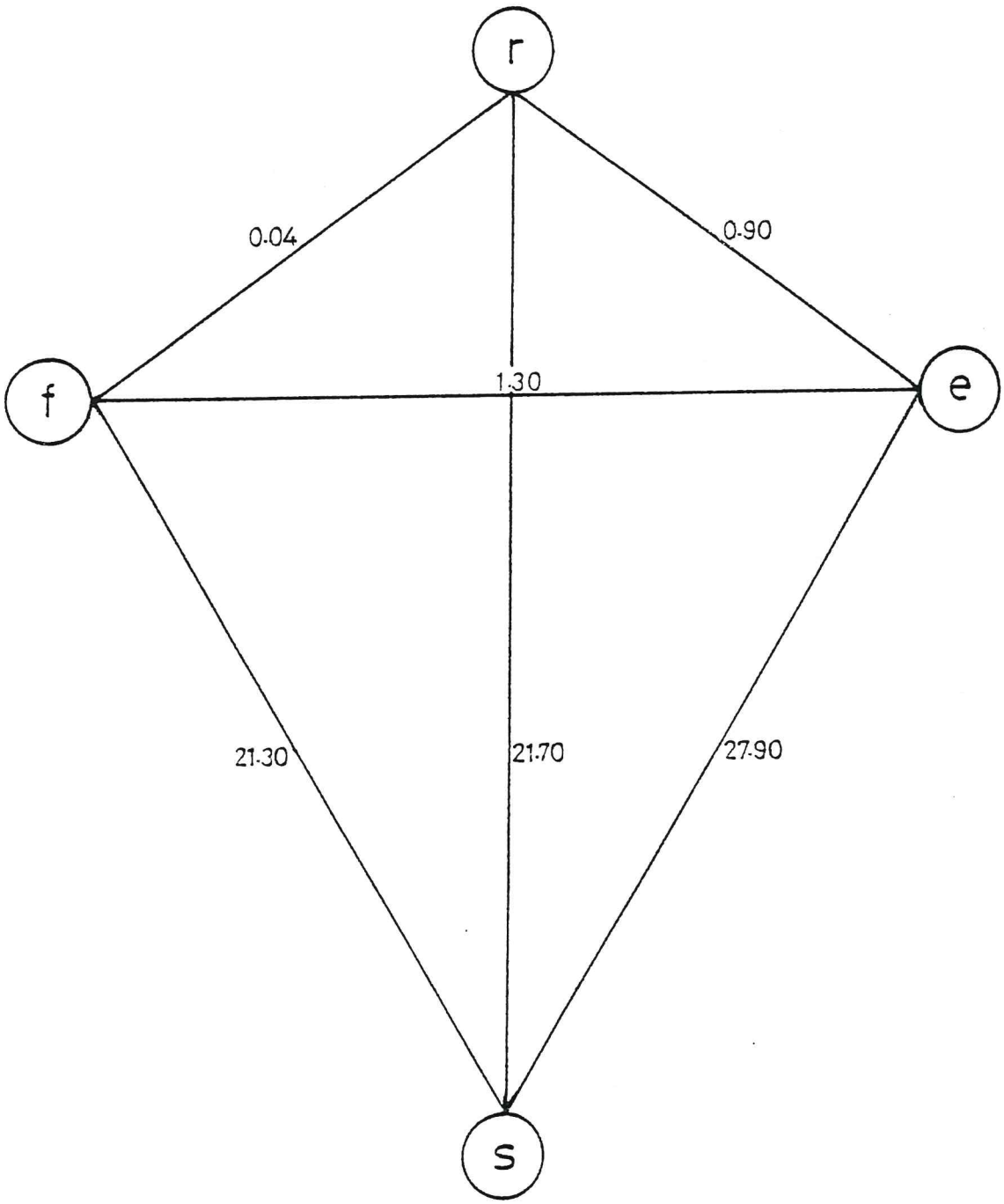
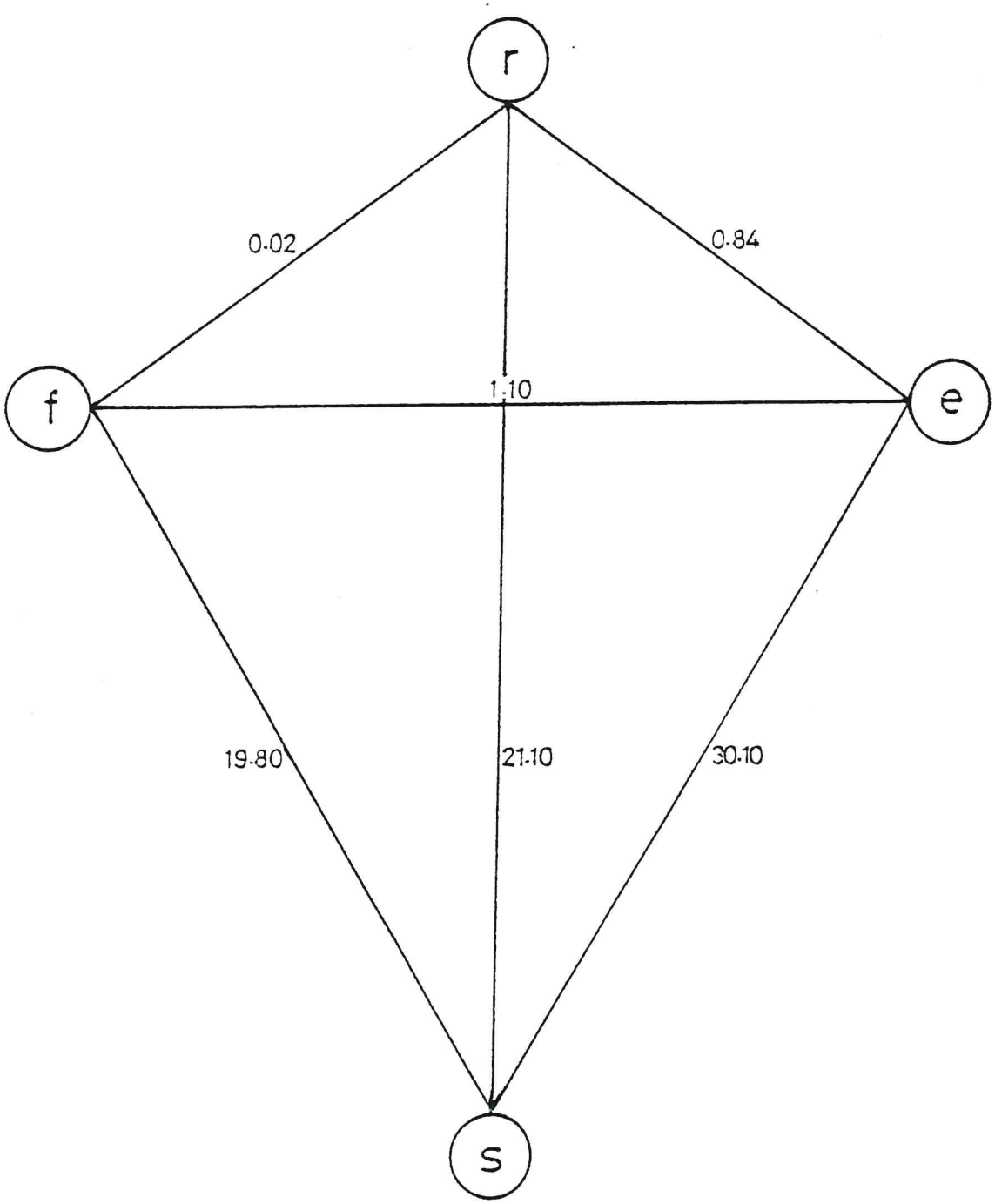


Figure 2.5 Mahalonobis distance relationship for the two ratios (E/F, F/C) among the four different locust populations of, *Schistocerca gregaria*. f= Red sea field population; r= Red sea lab. population; e= Ethiopian population and s= Kalahari field population



effective in population discrimination. The width of the head capsule (C) and the length of the elytron (E) shows higher weights in differentiating the four locust populations.

B. Comparisons with respect to location and sex

In the univariate analysis using the ANOVA test, means of the variable characters for the different individuals representing the four populations were significantly different (Table 2.6). The mean values of the southern males and females showed that the males had smaller values than the females for E, F, C measurements, but the mean values for the E/F and F/C ratios were approximately the same.

The coefficients of variances also indicated significant differences between the different forms for the three measured characters and the two ratios ($P< 0.0001$) (Table 2.7). The highest C.V. was for the length of the femur (40.18%) and the length of the elytron (38.49%), while the lowest C.V. was the E/F ratio (33.49%).

Multivariate analysis indicated that the two sexes of the four population were significantly different from each other ($P< 0.0001$). The first characteristic root for the three measured characters was 9.05 and the corresponding canonical

Table 2.6 Mean values and standard errors of the three characters for males and females from the four desert locust populations, *Schistocerca gregaria*

Form/ Character	E	F	C	E/F	F/C
r Male	53.61 ±0.39 c	24.64 ±0.18 c	6.95 ±0.08 a	2.17 ±0.01 c	3.54 ±0.03bc
r Female	61.45 ±0.63 a	27.75 ±0.37 a	7.72 ±0.08 b	2.21 ±0.02abc	3.59 ±0.03 b
s Male	48.57 ±0.46 c	23.89 ±0.23 d	5.77 ±0.04 c	2.03 ±0.02 d	4.13 ±0.03 a
s Female	56.97 ±0.37 b	28.04 ±0.27 a	6.68 ±0.06 f	2.03 ±0.01 d	4.19 ±0.03 a
e Male	52.03 ±0.36 d	23.33 ±0.18 d	6.76 ±0.05 fc	2.23 ±0.01 ab	3.45 ±0.02dc
e Female	58.31 ±0.46 b	25.94 ±0.28 b	7.48 ±0.06 c	2.24 ±0.02 a	3.46 ±0.03dc
f Male	53.81 ±0.75 c	24.71 ±0.19 c	6.90 ±0.04 dc	2.17 ±0.02 c	3.58 ±0.03 b
f Female	62.23 ±0.40 a	29.38 ±0.14 a	7.91 ±0.05 a	2.19 ±0.01 bc	3.59 ±0.03 b

* E: elytron length F: femur length C: head capsule width

* r: Ex-Addis population s: Southern population

* e: Ethiopian population f: Red sea population.

Table 2.7 Coefficient of variations in characters for males and females from the four desert locust populations, *Schistocerca gregaria*.

CHARACTERS	C.V. (%)	P > F
E	38.49	0.001
F	40.18	0.001
C	34.69	0.0001
E/F	33.49	0.0001
F/C	35.55	0.0001

* E: elytron length F: femur length C: head capsule width

variate explained 75.21% of the variability (Table 2.8). The second characteristic root was 2.95 with a corresponding canonical variate that explained 24.54% of the variability. The first characteristic root for the two ratios was 5.16 and the corresponding canonical variate explained 98.9% of the variability (Table 2.9). The second characteristic root was 0.06 and the canonical variate explained 1.1% of the variability. The respective canonical correlations were highly significant ($P < 0.0001$).

Different forms of the four different populations are shown in a canonical discriminant space for the three measured characters (Fig.2.6). There are differences in the morphology within the southern populations. The females are grouped in group 1 while the males are grouped in group 2. The three northern populations (males and females) are grouped in group 3. The canonical discriminant space for the two ratios showed that the females and the males of the southern populations have the same ratios (group 1) and that they are separated from the northern population (group 2) (Fig. 2.7).

The Mahalanobis distance between the centroids of the males and females of the four populations for the three measured characters and for the two ratios are shown in Table 2.10 and 2.11, respectively. The distances between the southern males and females are smaller compared to the distances between the

Table 2.8 Characteristic roots and proportion of the three measured characters for the males and the females from the four desert locust populations, *Schistocerca gregaria*.

Characteristic root (Eigenvalue)	Proportion (%)
9.05	75.21
2.95	24.54

Table 2.9 Characteristic roots and proportions of the two ratios for males and females from the four desert locust populations, *Schistocerca gregaria*

Characteristic root (Eigenvalue)	Proportion (%)
5.16	98.90
0.06	01.10

Table 2.10 Mahalanobis distances for the three measured characters between the centroids of the males and females from the four desert locust populations, *Schistocerca gregaria*

	r M	r F	s M	s F	e M	e F	f M	f F
r M	0	16.35	31.30	22.96	1.60	7.16	0.10	22.00
r F	16.35	0	77.76	32.73	25.42	3.26	16.55	0.63
s M	31.30	77.76	0	21.57	30.60	64.29	28.90	89.20
s F	22.96	32.73	21.57	0	32.33	34.71	20.66	37.82
e M	1.60	25.42	30.60	32.33	0	11.92	1.81	32.71
e F	7.16	3.26	64.29	34.71	11.92	0	7.70	5.94
f M	0.10	16.55	28.91	20.66	1.81	7.70	0	22.43
f F	22.00	0.63	89.20	37.82	32.71	5.94	22.43	0

* M: males F: females
* r: Ex-Addis poplation s: Southern population
* e: Ethiopian population f: Red sea population

Table 2.11 Mahalanobis distance for the two ratios between the centroids of the males and females from the four desert locust populations, *Schistocerca gregaria*

	r M	r F	s M	s F	e M	e F	f M	f F
r M	0	0.63	19.91	24.07	0.83	1.10	0.08	0.24
r F	0.63	0	18.15	21.76	1.21	0.92	0.36	0.13
s M	19.92	18.15	0	0.24	27.81	27.18	17.89	17.55
s F	24.08	21.76	0.24	0	32.48	31.62	21.79	21.28
e M	0.83	1.22	27.81	32.48	0	0.13	1.15	1.18
e F	1.10	0.92	27.18	31.62	0.13	0	1.27	1.12
f M	0.08	0.36	17.89	21.77	1.15	1.27	0	0.63
f F	0.24	0.13	17.55	21.28	1.18	1.12	0.06	0

* M: males F: females
* r: Ex-Addis population s: Southern population
* e: Ethiopian population f: Red sea population