GENETIC VARIATION IN WILD KENYAN POPULATIONS OF THE MEDITERRANEAN FRUIT FLY *CERATITIS CAPITATA* WIED.

BY

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A thesis submitted in partial fulfilment for
the degree of Master of Science
in the University of Nairobi

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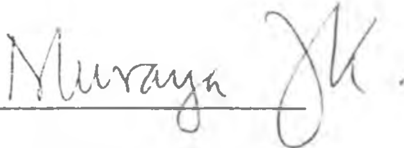
Nairobi - Kenya.

1989

DECLARATION

I, J.K. Muraya, hereby declare that this is my own work and has not been presented for a degree in any other University.



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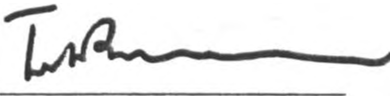
This thesis has been submitted for examination with our approval as University supervisors.



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ACKNOWLEDMENTS

I would like to most sincerely thank Dr. T.K. Mukiama my first supervisor for his guidance and patience during the preparation of this work, and for availing to me his chemicals and equipment.

I also wish to thank Prof. R.W. Mwangi my second supervisor for his valuable advice and guidance. Special thanks to Prof. J.O. Kokwaro of Botany Department for his administrative help and many acts of kindness during the course of my study in the department.

To Dr. Osir of I.C.I.P.E, and Dr. Makawiti of Biochemistry department, University of Nairobi I am grateful for the good advice they gave to me. I am indebted to Mr. S.N. Karanja and Mr. H. Khayo of Botany department for their help with photography, and Mr. J. Amoshe of Zoology department for helping with dissections. Thanks are due to the Kenya Coffee Research Foundation for availing coffee samples from their substations in western Kenya, to the German Academic Exchange Program (DAAD) and to the University of Nairobi for financial support.

Lastly but not least I wish to thank Gathoni for her encouragement and understanding patience during the preparation of this work.

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ABSTRACT

Over the period November 1987/ June 1988, a survey of the Mediterranean fruit fly (medfly), *Ceratitis capitata* occurrence was conducted. Samples of ripe coffee were collected from 8 districts. In 7 districts, the fly was found present and sympatric with *C. rosa* and *C. nigra*.

The electrophoretic variation of 6 enzymes, β-Hydroxyacid dehydrogenase (β-HAD), Aldehyde oxidase (AO), Malate dehydrogenase (MDH), α-Glycerophosphate dehydrogenase (α-GPDH), Alcohol dehydrogenase (ADH) and Glutamate oxaloacetate transaminase (GOT) were studied in Kenyan strains of the medfly. The medflies were obtained from ripe coffee berries. The berries were kept in paper cartons lined with sawdust in the insectary and regularly inspected for presence of pupae for 2 weeks. Adults emerged in cages within the next 14 days, upon which they were used for gel electrophoresis. The MDH locus was monomorphic, while only two alleles per locus were detected at AO, ADH, α-GPDH and GOT loci. Five different alleles were identified at β-HAD locus. Mean heterozygosities for the two adequately sampled areas, Machakos and Kabete were 0.118 and 0.117 respectively. The allele frequencies were in agreement with Hardy-Weinberg expectations except the β-HAD locus in Kabete, Embu and Murang'a where significant deviations were observed.

Chromosomal constitution was determined by observing salivary gland chromosomes and brain ganglia cells in larvae and adult flies respectively. Rearing of the medflies was done in

perspex cages. Fruit materials were offered for ovipositing to newly hatched flies. Ripe banana was found to be the most attractive medium to oviposit in. At most, two generations of medflies were maintained in the laboratory using banana fruits as oviposition medium.

INTRODUCTION

The Mediterranean fruit fly Ceratitis capitata Wied. is a member of the order Diptera, family Tephritidae. The family has about 4,000 members in temperate, subtropical, and tropical areas (Le Pelley, 1968). Other members of the genus sympatric with C. capitata in Kenya are C. nigra (Trirhithrum coffeae) and C. rosa (Waikwa, 1977). Univoltine tephrids have a winter diapause and inhabit colder areas while multivoltine tephrids have no obvious diapause and inhabit warmer areas.

The Mediterranean fruit fly (medfly) is considered to have originated from tropical West Africa from where it spread to the North. It invaded Spain and later spread to European Mediterranean countries and the Middle East. It appeared in Australia, Hawaii, Costa Rica, and South America spreading to Western South America through Central America and finally to Southern Mexico (Hagen et al.1981).

The adult C. capitata measures about 4 mm and has the following distinguishing features: the abdomen is characterised by black yellow crossbands, wings have brown ochraceous markings and eyes are wine coloured (Waikwa, 1977). When reared at 23.8 ± 2°C, C. capitata completes its life cycle in a minimum of 20 and a maximum of 33 days. The female oviposits eggs in the host fruit where they hatch into larvae and undergo four instar stages before pupating. The larvae fall off from the fruit into the soil, pupate, and later emerge as adults. The medfly usually

has between 8 and 10 generations in one year.

The medfly is a serious pest of soft fruits in most parts of the world. Over 250 fruit and nut trees and vegetables have been reported as hosts and 40 of them are either heavily or generally infested (Kourti et al., 1985). Among the infestable fruits are peach, pear, plum, pawpaw, loquat, banana, orange and pineapple. Known wild hosts of C. capitata are Carissa edulis, Dovyalis caffra and species of Boschia, Drypetes, Brucea, Rawsonia and Acokanthera genera (National Museums of Kenya, Natural Sciences Division, 1987). In Kenya, the medfly mainly infests coffee. The coffee industry however does not consider it a serious pest (Abasa, 1972) but the Citrus industry does. Fruits are a highly priced commodity and in Kenya, Citrus is the largest fruit crop by tonnage after pineapple and banana (Report to Director of Agriculture, 1982).

Major advances in insect control in the 20th century have been host plant resistance, development of synthetic organic insecticides, sterile insect technique and integrated pest management. Undesirable consequencies arising from the use of insecticides include disturbance of preditor-prey relationships sometimes benefiting the pest, residues persisting in food, feedcrops, soil and animals, and resistance to the insecticides. Todate, 447 species of insects and mites have shown resistance to one or more insecticides, excluding 171 species of veterinary and medical importance, and 12 beneficial insects (Georghiou, 1986). Of the agriculturally important insects, 21 species have

demonstrated an ample capacity for resistance. Among the tephritids, limited ability to resist D.D.T., dieldrin, and organophosphates has been observed. Selection of laboratory strains of _C_. _capitata_ has provided evidence for resistance. Biochemical investigations have revealed the presence of organophosphate metabolizing esterases, and of acetylcholinesterases with lower sensitivity towards inhibitors. This has not however been demonstrated in natural medfly populations (Georghiou, 1986).

For the last four decades, emphasis in insect control has shifted to insect pest management using integrated approaches. Since the successful control of the screwworm fly (_Cochlimyia hominivorax_) in the U.S.A (Baumhover, 1966), and the melon fly _Dacus dorsalis_ (Steiner _et al_., 1965), such approaches have been tried on the Australian sheep blow fly _Lucilia cuprina_, the tse-tse fly (_Glossina_) species and _C_. _capitata_ amongst others. Most emphasis is on the use of sterile insect release technique. Generally, pest management techniques are aimed at keeping pest levels below an economic threshold. Such management must be based on a thorough and comprehensive knowledge of the subject species.

There is a wealth of information published regarding the biology, physiology, behaviour and ecology of the medfly. Given its economic importance, it is poorly studied from the stand point of genetics and especially population genetics. Moreover, most genetic studies have been on Mediterranean populations.

CHAPTER 1

1 LITERATURE REVIEW

1.1 ECONOMIC IMPORTANCE

The earliest documented report on C. capitata in Kenya is by Graham (1959), although earlier specimens are displayed in the National Museum. Later reports have mainly dwelt on the medfly and its association with coffee. The chief coffee growing areas in Kenya are the highlands east and west of the Rift Valley.

The stinker coffee bean was first observed in Kenya by Northmore (1969) and was subsequently linked to the presence of insect larvae in the ripening coffee berries (Gibson, 1970).

The medfly is known to cause premature coffee drop (Ritchie, 1928) but its importance in this respect is uncertain. In a study by Abasa (1972) it was reported that the premature drop in coffee due to the medfly was insignificant. In the first comprehensive study of C. capitata in Kenya, Waikwa (1977) noted that only 7% of the coffee berries dropped prematurely and he further attributed a significant 40% of this "drop" to the medfly.

1.2 ECOLOGICAL ASPECTS

Detailed information on the determinants of fecundity and developmental rates of fruit flies is available (Christenson and Foote, 1960; Bateman, 1972). The determinants include light, food, temperature, moisture and natural enemies.

In Kenya, the medfly is known to have peak breeding seasons corresponding to peak coffee ripening seasons i.e. July-September and February-April (Waikwa, 1977; 1978). The chief controlling factor in breeding is the presence of host fruits. A continuous supply of host fruits facilitates the presence of fruit flies throughout the year. Different fruits have differing attractiveness as oviposition media (Waikwa, 1979). In a detailed documentation of the life cycles of fruit flies in coffee farms, Waikwa (1977) observed that both pupae and larvae had a diurnal periodicity and that larval stages were longer in green fruit or when temperatures were cool.

C. capitata is a sympatric infestant of coffee with C. (= Pterandrus) rosa and C. nigra. Besides coffee, it infests commercial oranges and it is the dominant fly in the main arabica coffee growing areas (Mukiama, 1985).

In a review on the ecology of fruit flies (Bateman, 1972), it was noted that medflies are strong fliers with some being captured 3 km away from the points of release. The review further observes that fruit flies are attacked by Hymenoptera. Steyn (1955) indicated that Pheidole megacephala preys on fruit fly larvae. More reports on fruit fly parasitoids are given by Michelakis (1986), Serit et al., (1986) and Lindquist (1986). Several symbionts including Pseudomonas savastanoi and Phytomonas mutabilis have been cultured from the guts of fruit flies and are thought to produce nutrients absent or in low quantities in diets of host insects (Allen and Ricker, 1932).

1.3 LABORATORY COLONIZATION

The control of dipteran pests using biological methods usually involves releasing large numbers of insects. There is therefore need to colonize and develop media for mass production and maintenance of such insects in the laboratory.

Larval media used in laboratory cultures and mass production of C. capitata are mostly based on Finney (1956). The basic ingredients are yeast hydrolysate and dehydrated carrot powder which provide all the nutrients for larval growth (Tanaka et al., 1969). The other ingredients are hydrochloric acid (HCl), sodium benzoate and a moisture control agent, Gelgard. The media are spread 2 cm deep in trays. The eggs are then introduced and larvae are washed off 5 or 6 days later. Besides C. capitata, efficient rearing systems have been developed for the melon fly (Schroeder et al., 1970), olive fruit fly (Tzanakakis and Economopoulos, 1967) and the apple maggot larvae (Prokopy, 1967).

The disadvantages of fruit as a larval medium are lack of availability of the fruit all the year round and inability to regulate oviposition. Only a limited number of larvae can complete their life cycle in a single fruit.

Male size is known to increase mating propensity in C. capitata. Mating propensity increases with the size of the male and this has been associated with loudness of sounds made during courtship (Stanland et al., 1986). Nakagawa et al.,

(1970) have reported that medfly females respond to trimelure, medlure and angelica seed oil male lures in absence of sexually mature males. Shade and apple baits have been observed to increase longevity and fecundity of the apple maggot in laboratory cultures (Prokopy, 1967b). Allelochemicals potassium and tannin are known to enhance larval growth in the olive fruit fly (Manoukas, 1986).

Efficiency of rearing mainly concentrates on optimization of fly density in cages, egg density in larval diet and design and construction of equipment used in the phases of rearing (Bruzzone, 1986; Tsitsipsis, 1986). Optimum rearing temperature is reported to be 25°C. Hakim amd Basilly (1986) have reported on efficiency and costs of several rearing media for C. capitata. Yeast hydrolysate and carrot powder are the most expensive ingredients of the media and varying quantities were used as well as different sources of sucrose.

1.4 CHROMOSOMAL POLYMORPHISM

Chromosomal polymorphism is widespread and especially well studied in Drosophila (Kastritsis, 1969; Dobzhansky and Pavlovsky, 1962; Ehrman, 1963) and in grasshoppers (Jackson and Cheung, 1967; Hewitt and Schroeter, 1968; Hewitt and John, 1971; 1972). These studies have shown the presence of inversion polymorphism and supernumeraries.

The significance of evaluation of chromosomal status is evident in light of the effectiveness of control methods employed

for dipterans and possible detection of different races within the same species. In controlling the screwworm (Cochlomyia hominivorax), fluctuations in success were due to presence of chromosomal polymorphism. Field populations had different karyotypes which though geographically overlapping were reproductively isolated (Richardson et al.,1982). Incompatible geographical races of Rhagoletis cerasi have also been observed (Boller, 1976; Berlocher and Bush, 1982).

The Ceratitinae group is known to have a fairly stable number of chromosomes (Boyes and Brink, 1965). C. capitata has six pairs of chromosomes occurring in pairs, two are metacentric, two submetacentric and two are subtelocentric including the sex determining Y chromosome (Radu et al., 1975; Bedo, 1986). Manso and Lifschitz (1986) have observed a minute heterochromatic supernumerary chromosome in cerebral ganglia cells of both sexes. However, only males transmit it to the progeny. Chromosome three centromere was also noticed to be variable in location.

1.5 GENETIC VARIATION IN CERATITIS SP.

Electrophoretic studies on the Ceratitinae group are few compared to other dipterans (Kasritsis, 1969; Berger, 1971; Marinkovic and Ayala, 1971). Most of the documented genetic variation in the group is morphological. Hakim and Awad (1986) studied pupal colour mutants and showed that colour differences

were due to dominant and independently inherited genes with cummulative effects. Since 1973, several other mutants have been described. Presently; 11 eye colour mutants, 3 eye shape mutants, 7 pupal mutants and 7 imago mutants have been described (Rossler, 1979; Saul and Rossler, 1984; Lifschitz, 1985; Manso et al., 1986)

Using samples of C. capitata from Kenya, Libya, Sardinia and Procida islands, Gasperi et al. (1986) investigated more than 21 enzyme loci. In Kenya, C. capitata was found to be very similar to C. rosa when their heterozygosities, allelism, and proportions of polymorphic loci were compared. Five of the biochemical loci were fully diagnostic between the two species.

The above study revealed that alcohol dehydrogenase (ADH) enzyme system had two isoenzymatic forms. A third and rare allele was present in the southern Africa populations. The earliest report on the Adh locus in C. capitata is by Heemert and Brink (1980). More recently, Krietman and Aguade (1986) observed that the Adh locus in Drosophila melanogaster was unexpectedly highly polymorphic. In some species of Rhagoletis and Anastrepha which belong to the same taxonomic group as C. capitata, ADH is controlled by two genes at two loci (Berlocher and Bush, 1982; Matioli et al., 1986). In the medfly Adh locus is also controlled by two loci on chromosome 2 (Zapater and Robinson, 1986).

Kourti and Loukas (1986), studied 25 enzyme loci including

Adh in seven populations. Compared to five Mediterranean populations, those from southern Africa and Reunion had higher heterozygosity and polymorphism values and thus higher levels of genetic variation. In Kenya medflies have been reported as having 4 electromorphs at the Adh locus. The commonest allele occurred in very high relative frequency and the proportion of heterozygous genome was very small at this locus (Mukiama, 1985).

Glutamate oxaloacetate transaminase (GOT) has been found to be monomorphic in Mediterranean populations of C. capitata. A second and rare allele was observed in southern Africa and Reunion populations (Kourti and Loukas, 1986).

In a study of the sympatric C. capitata and C. rosa from four distinct regions, (Gasperi et al., 1986) the aldehyde oxidase (Ao) locus was reported as fully diagnostic between the two species in Kenyan populations. Both species displayed the allele as monomorphic with the allele for C. capitata having a slower mobility.

α-Glycerophosphate dehydrogenase (α-Gpdh) has been observed to have two alleles in a lone Reunion population. Mediterranean populations were monomorphic for the allele α-Gpdh (Kourti and Loukas, 1986). However four alleles were observed in medflies from Kenya whereas C rosa was noted to be monomorphic for the allele α-Gpdh (Gasperi et al., 1986).

For the β-hydroxyacid dehydrogenase (β-Had) locus, four alleles and several nulls have been observed (Mukiama, 1985) in Kenyan populations of the medfly.

Generally Mediterranean populations have been shown to be highly monomorphic (mean heterozygosity ($\bar{H}$) = 0.043)). In contrast, populations from Reunion and southern Africa are highly polymorphic ($\bar{H}$ = 0.15). When the populations of the above named regions were considered together and some parameters determined, 11% of the loci were established to be polymorphic and 4.3% of the loci in an individual heterozygous (Kourti and Loukas, 1986). Morgante et al. (1981) found a mean heterozygosity value of 3% in Brazillian populations of the medfly. Dacus olea, D. antiqua, Rhagoletis pamonela and R. completa are monophagous and infest olive trees amongst others. Their heterozygosities are 25.7% (Loukas et al., 1985), 9.0%, 18.1% and 7.5% (Berlocher and Bush, 1982) respectively.

Since the early estimates of heterozygosity and polymorphism (Hubby and Lewontin, 1966; Selander and Yang, 1969) many papers (Richmond, 1972; Yoshiko and Kojima, 1972; Barker and Mulley, 1976; Black and Krafsur, 1986) have shown that up to 40% of the loci in a population may be polymorphic and that 12-15% of the loci are heterozygous in natural populations of plants and animals. The corresponding values for medflies are consistently lower. Fuerst et al. (1980), have predicted that populations which have maintained large numbers in the recent past have a U-shaped allele frequency distribution, while those populations which have gone through a bottleneck exhibit a bimodal allele frequency distribution. The southern Africa medfly populations have a U-shaped allele frequency distribution and the

Mediterranean populations have a bimodal allele frequency distribution.

1.6 OBJECTIVES

The objectives of this study were:

1 To colonise C. capitata in the laboratory.

2 To characterise the chromosomal constitution of local strains of C. capitata.

3 To determine the genetic variation at six enzyme loci in C. capitata. These are,

Alcohol dehydrogenase

α-Glycerolphoshate dehydrogenase

Aldehyde oxidase

Glutamate oxaloacetate transaminase

β-Hydroxyacid dehydrogenase

Malate dehydrogenase.

CHAPTER 2

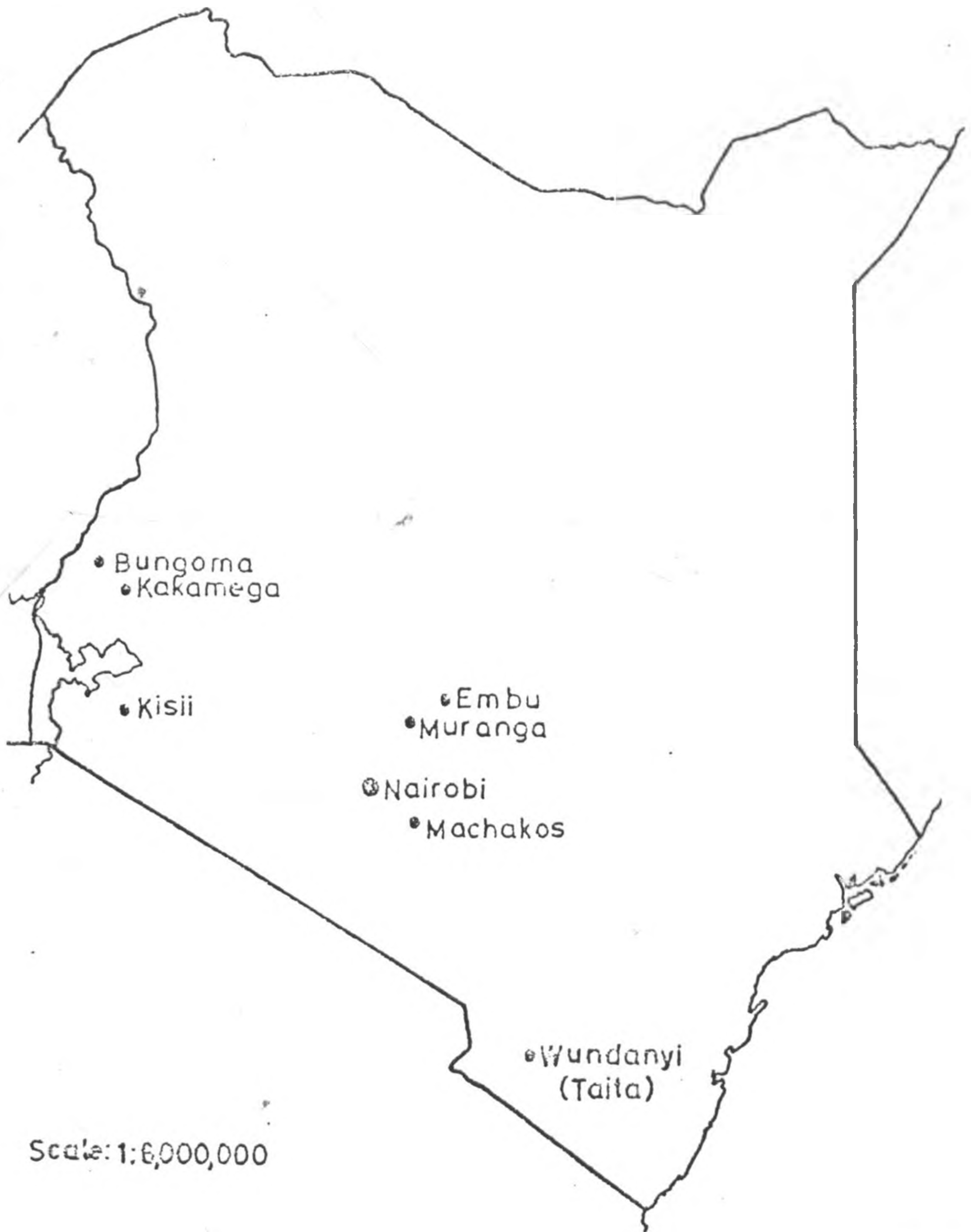
2 MATERIALS AND METHODS

2.1 STUDY AREAS

All the medflies used in this study were collected from Nairobi, Machakos, Embu and Muranga districts within the highlands east of the Rift Valley; Kakamega and Bungoma districts in western Kenya and Wundanyi in Taita Taveta district which adjoins Mt. Kilimanjaro. The coffee varieties grown in these areas are Coffea arabica on highlands east of the Rift Valley and C. robusta west of the Rift Valley respectively.

In Nairobi province, medflies were obtained from the University farm at Kabete 10 km north-west of Nairobi. Most of the cultivated land in this area is under coffee. The area is densely populated and lies between 1,500 and 2,000 m above sea level. Rainfall varies from 760-1015 mm annually and falls in two seasons: March to May and October to December. Temperatures range from an average minimum of 10°C to a maximum of 26°C. Black cotton soils occur widely in the area. Fruits grown in this area include orange, peach, plum, tangerine, pear, banana, loquat and pineapple.

Machakos district lies between 1,500 and 2,500 m above sea level and receives an annual rainfall of 760-1015 mm. Soils are mostly red clays and sandy loams of volcanic or basement complex origin. Subsistence crops grown in the area include maize, millet and pulses, while fruits include pawpaw, loquat, lime, orange, avocado, green mango and passion fruit. The natural vegetation


Bungoma
Kakamega
Kisii
Embu
Muranga
Nairobi
Machakos
Wundanyi
(Taita)
Scale: 1:6,000,000

varies from wooded grassland to grassland and the district is moderately populated.

Embu district is situated between 1,500 and 2,500 m above sea level and annually receives 760-1015 mm of rainfall. Mean temperatures vary from 14°C-30°C. Dark red friable clays and soil with deep humic top layer are the commonest in the area. Maize, millets and pulses are the subsistence crops grown while fruits include orange, lime, pawpaw, green mango and guava. Most of the natural vegetation is forest and wooded grassland and the population varies from moderate to very dense on the higher areas.

For Muranga district, the mean annual temperature varies from 10°-26°C. Most of the district receives between 1270 and 1525 mm of rainfall annually. Dark red friable clay soils are most frequent in this district which lies between 1,500 and 2,500 m above sea level. Cereals grown include maize and millets while fruits include pear, plum, green mango, banana and pineapple orange, loquat and guava as well as pulses. The population density varies from sparse to dense in this area whose natural vegetation is mostly bush or wooded grassland in the lower limits and forests within the higher reaches.

Most of Taita Taveta district is located below 1,000 m above sea level although the highlands rise upto 2,500 m. Mean annual temperatures are between 14°C and 26°C. This area has red sandy loams as the dominant soil type and the highlands receive 1275-1525 mm of rainfall annually. Amongst the crops grown in the district are maize, millets, coffee and sisal.

Situated on the western side of the Rift Valley, Kakamega and Bungoma districts are in the same climatic zone. Both lie at 1,000-1,500 m above sea level and receive a rather high (1270-1525 mm) rainfall annually. Mean temperatures in both areas vary from 14°C to 30°C. Dark brown sandy loams and yellow-brown sandy soils are the dominant soil types. Crops grown include maize, millets, pulses,cassava, mango, orange, banana, guava and loquat. The population density varies from a sparse 10 persons to a very dense 950 persons per square kilometre.

2.2 LABORATORY HANDLING

The mature ripe handpicked coffee berries were divided into single samples of 1 kg each. The samples were spread out in paper cartons lined with sawdust and kept in an insectary at 22°C-25°C. On a regular basis, the cartons were inspected for pupae until the coffee was unlikely to contain any more. Usually, this lasted for 2 weeks.

The pupae collected were placed in cages until the adult flies emerged. The emerged adult medflies were either used for rearing, used immediately for electrophoresis, or deep frozen for later use.

2.3 LABORATORY COLONIZATION

The medflies were reared in perspex cages measuring 27 cm by 30 cm by 30 cm with an opening 10 cm by 12 cm on one side. A flannel cloth was fixed over the opening to keep out other flies and also provide access inside the cage.

The flies were fed on a 3:1 sucrose yeast hydrolysate medium provided in a petri dish. Water was provided to the flies through a mass of cotton wool soaked in a petri dish half full of water.

Several fruit materials including loquat, orange, banana, mango, pawpaw and Kei apple were offered each at a time for oviposition. Before 5 days, the fruits were removed and kept in specially designed containers. Each container had a netted top lid and a perforated bottom which was layered with a larval medium consisting of :

HCl (36%)	36 ml
Yeast hydrolysate	105 g
Sodium benzoate	8.3 g
Carrot powder	420 g
Water	2.5 l

Larvae ready to pupate left the containers through the perforations and fell on to a collecting tray covered with vermiculite.

Net-hole fabrics were also provided for the flies to oviposit through. The fabrics were laced with fruit materials and egg collectors placed bellow them to collect any laid eggs.

2.4 ELECTROPHORESIS

All the enzymes were run on a vertical polyacrylamide gel system described by Hames and Rickwood (1981).

2.4.1 BUFFERS

The buffer systems used in this study are modified from those described by Hames and Rickwood (1981).

(i) Stock reservoir buffer.

The buffer was made of 3.03 g tris and 144.4 g glycine dissolved in distilled water and made to 1 litre with distilled water. The final pH was adjusted to 8.3 using tris. This solution was stored in a refrigerator at 4°C and was used straight.

(ii) Stock resolving gel buffer.

The buffer was made by using 36.3 g tris and 48 ml 1M HCl which were mixed and brought to 100 ml final volume with distilled water. The final pH was adjusted to 8.8 by adding HCl, filtered through Whatman No. 1 filter paper and stored at 4°C.

(iii)Stacking gel buffer.

In 40 ml distilled water, 6.0 g tris was dissolved and titrated to pH 6.8 with 1M HCl (about 48 ml) and brought to 100 ml final volume with distilled water. The solution was filtered through Whatman No.1 filter paper and stored at 4°C.

2.4.2 GEL PREPARATION

For this study, 7.5% acrylamide gel was used. Acrylamide-bisacrylamide was prepared by dissolving 30 g of acrylamide and 0.8 g bisacrylamide in a total volume of 100 ml distilled water. The solution was filtered through Whatman No.1 filter paper and stored at 4°C in a dark bottle. Only enough acrylamide-bisacrylamide stock solution to last 1-2 months was

prepared.

The reagents and the proportions used were:

Acrylamide-bisacrylamide	7.5 ml
1.5% ammonium persulphate	1.5 ml
Resolving buffer	3.75 ml
Distilled water	16.95 ml
N,N,N',N'-Tetramethylethylene diamine (TEMED)	0.015 ml

The gel mixture except TEMED was prepared in a small thick walled flask and degassed for one minute using a vacuum pump.

The correct volume of TEMED was then added and the mixture gently but rapidly mixed by swirling. Immediately, the mixture was poured between two glass plates. The glass plates were 16.0 cm by 14.5 cm and 0.3 cm thick, and one plate was notched 1.5 cm deep on the top side. The plates were held apart by polyvinyl chloride (PVC) spacers 0.3 cm, thick which were sealed by coating with Vaseline petroleum jelly before assembly. The spacers were placed down on the two vertical sides and on the bottom side and held in place by one piece metal clamps. The spacer on the bottom side was removed after the gel had set and prior to electrophoresis.

Once the gel solution was poured to the appropriate depth using a syringe, it was overlayered with water to a depth of 0.5 cm. The solution was left undisturbed for 10-30 minutes within which it polymerised. The water overlay was then poured out and a stacking gel mixture 2 cm deep poured in.

The stacking gel mixture consisted of:

Acrylamide-bisacrylamide	2.5 ml
Stacking gel buffer	5.0 ml
1.5% ammonium persulphate	1.0 ml
Distilled water	11.3 ml
TEMED	0.015 ml

A 13 toothed comb was placed on the mixture before it set to provide 0.5 cm wide and 1.5 cm deep wells for loading samples into. The solution was similarly let to set for 10-30 minutes without disturbance. The comb was then slowly and carefully removed.

2.4.3 SAMPLE PREPARATION

Newly emerged or deep frozen whole insects were separately ground in crucibles in a drop of glycerol and water in the ratio 1:1 to make a crude homogenate. They were ground on ice blocks in order to maintain a low temperature to prevent enzyme denaturation.

2.4.4 SAMPLE LOADING AND ELECTROPHORESIS

The electrophoresis apparatus was the Studier-type gel apparatus made of perspex. The apparatus was made locally at the University of Nairobi science workshops.

The PVC spacer at the bottom of the glass plates was removed and the gel slab mounted in place on the electrophoresis apparatus. The gel slab assemblage was clamped into place using

metal clips with the notch on the glass plates aligned with the notch on the upper reservoir. A seal between the electrophoresis apparatus and the glass plate was achieved by coating the area around the apparatus notch with Vaseline petroleum jelly and then clamping the glass plates onto the apparatus. Reservoir buffer was then added to the lower reservoir.

The electrode buffer was introduced to the top reservoir and on the gel surface using a Pasteur pipette. Into each 0.5 cm wide well, 50 μl of the crude enzyme extract was carefully loaded using a microsyringe. One well was loaded with bromophenol blue dye as a marker.

A power supply (ISCO model 493) was then connected to the electrophoresis apparatus with the anode (+) connected to the bottom reservoir and the cathode (-) connected to the upper reservoir. The power supply was then connected to the mains, switched on and adjusted to deliver 20 mA for stacking. The gel apparatus was then transferred to a refrigerator and left to run for 30 minutes. The power supply was then adjusted to deliver 40 mA and left to run till the marker dye had migrated 10 cm from the point of origin (this usually took 4 hrs). After running sufficiently, the power supply was switched off and the gel slabs stained immediately for the appropriate enzyme.

The different enzymes were run using buffers which correspond to their respective peak activity pH as follows;

α-glycerolphosphate dehydrogenase pH	7.5
β-hydroxyacid dehydrogenase	8.0
Alcohol dehydrogenase	8.0

Glutamate oxaloacetate transaminase	8.0
Aldehyde oxidase	7.5
Malate dehydrogenase	7.5

2.4.5 STAINING

The staining methods used in this study are those described by Steiner and Joslyn (1979).

Stain buffers:

The stain buffers used were tris - HCl and phosphate buffer.

(i) The tris - HCl buffer pH 7.5 was made by dissolving 12.2 g tris in 7.5 ml concentrated HCl and brought to 1 litre with distilled water.

(ii) Tris - HCl buffer pH 8.0 was made by dissolving 24.2 g tris in 11.8 ml conc. HCl and brought to 1 litre with distilled water.

(iii) Phosphate buffer was made by mixing monobasic and dibasic sodium phosphates. 13.2 g of monobasic sodium phosphate was dissolved in 500 ml water to make solution A. Solution B was made by dissolving 26.81 g dibasic sodium phosphate in 500 ml distilled water.

To make a basic pH phosphate buffer, 10 ml of solution A was mixed with 50 ml solution B and 40 ml distilled water.

2.4.5.1 STAINING PROCEDURES

The constituents of each staining mixture for each enzyme were as follows.

(i) Alcohol dehydrogenase:

Stain buffer pH 8.0	35 ml
Ethanol	5 ml
Nicotinamide adenine dinucleotide (NAD)	20 mg
Methyl thiazolyl tetrazolium (MTT)	10 mg

The gel was incubated in the stain mixture for one hour at 37°C in the dark. 5mg of phenanzine methosulphate (PMS) was then added and the gel incubated for 1 hour more. The stain was removed and a wash solution (6 parts absolute methanol and 5 parts water) added.

(ii) Glutamate oxaloacetate transaminase:

Aspartic acid	130 mg
α-Ketoglutaric acid	85 mg
Pyridoxol-5'-phosphate	1.5 mg
0.1M phosphate buffer pH 7.4 (adjusted using 5N sodium hydroxide)	60 ml

The gel was then incubated for 30 minutes in the dark at 37°C before adding Fast Blue RR salt. The gel was further incubated for 2 hours, the stain removed and wash solution added.

(iii) α-Glycerolphosphate dehydrogenase:

Stain buffer pH 7.5	35 ml
α-Glycerolphosphate	50 mg
MTT	10 mg
NAD	20 mg

The stain mixture was poured over the gel and incubated at 37°C for 30 minutes in the dark. 5mg of PMS was then added and the gel incubated for a further 1 hour in the dark, the stain

removed and wash solution added.

(iv) Malic dehydrogenase:

Stain buffer pH 7.5	35 ml
MTT	10 mg
D-L malate pH 7.4	0.268 g

The mixture was poured over the gel and incubated for 30 minutes in the dark at room temperature. 5 mg of PMS was added to the mixture and incubated for 30 minutes more in the dark. The stain mixture was drained off and the wash solution added immediately after.

(v) β-Hydroxy acid dehydrogenase:

Stain buffer pH 8.0	35 ml
D-gluconic acid	100 mg
MTT	10 mg
Sodium chloride	100 mg
Magnesium chloride	0.2 ml(2.03g/100ml water)
NAD	20 mg

The mixture was poured over the gel and incubated in the dark at 37°C for 30 minutes, 5 mg of PMS was added and the mixture incubated at 37°C for 2 hours more in the stain before adding the wash solution.

(vi) Aldehyde oxidase:

Stain buffer pH7.5	35 ml
Benzaldehyde	0.5 ml
MTT	10 mg

The mixture was poured over the gel and incubated for 1 hour at 37°C. 5 mg of PMS was added and the gel incubated for

a further 30 minutes, the stain removed and the wash solution added.

All the slab gels remained in the wash solution for at least 30 minutes before recording the resultant enzyme bands. Photographs were taken using Kodak Panatomic X film. The gels were laid directly onto an illuminator with an opal white screen. Slab gels were stored in plastic bags containing 7% acetic acid.

2.5 NOMENCLATURE

The nomenclature used for enzymes in this study was adopted from Ayala et al.(1972). Abbreviations were designated for each enzyme and the same abbreviations used to represent the genes coding for the enzymes. When several forms of the same enzyme exist, each controlled by a different gene locus, a hyphenated numeral was added to the abbreviation of the enzyme. The enzyme with the least anodal migration was called one, the next two,and so on.

The most common allele was given the arbitrary value of 100 and an allele coding for an enzyme which migrates 5 mm more towards the anode was called 105. An allele whose enzyme migrates 5 mm less was designated 95. Alleles are written as superscripts over the symbol representing the locus e.g. Adh^{100} and Adh^{95} are two alleles at the alcohol dehydrogenase locus.

2.6 CALCULATION OF GENE FREQUENCIES

Gene frequencies were worked out as follows: In a population sample of 40 individuals, if when assayed 20 were found

homozygous for the allele β-Had^{100} (i.e. genotype 100/100) and the other 20 heterozygous for the alleles β-Had^{100} and β-Had^{95} (genotype 100/95), then since in a diploid individual each gene is represented twice, a total of 80 genes have been sampled. The frequency of the allele β-Had^{100} is (20x2)+(20x1)/80 =0.75 while that of the allele β-Had^{95} is (20x1)/80 =0.25, (after Hubby and Lewontin, 1966).

2.7 CALCULATION OF HETEROZYGOSITY VALUES

Heterozygosity values at the various enzyme loci in samples from different localities were calculated after Nei and Roychoudhury (1974) as follows:

$H = \Sigma hi/r.$

where $hi = 1- x^2 i$,

r = number of loci examined and

x = frequency of the ith allele.

2.8 CALCULATION OF GOODNESS OF FIT

Each population was tested for agreement with the Hardy-Weinberg expectations by comparing the worked out and the tabulated Chi-square values. For every allele, the expected number of individuals was calculated thus: If the frequency of allele β-Had^{100} was 0.75 in a population sample of 40 individuals, the number of individuals expected to be homozygous for the allele is

$(0.75)^2 x40 = 22.5$ and

$(0.25)^2 x40 = 2.5$ for the β-Had^{95} allele.

A Chi-square value was then obtained using the formulae

$X^2 = (O-E)^2/E$. (Bailey, 1981).

where O = observed number of individuals

E = expected number of individuals.

The answer was compared to the tabulated Chi-square values at the appropriate degrees of freedom at $p = 0.05$.

2.9 CALCULATION OF GENETIC IDENTITY AND DISTANCES

Genetic identities and similarities for the different populations were computed using a BASIC program designed by Green (1979) with corrections by Hillis and Canatella (1983) and Green (1984) to accomodate small samples.

2.10 CHROMOSOME OBSERVATIONS

The medflies used for chromosome preparations were reared in the laboratory at 20°C 2°C and fed on a medium consisting of 33% yeast hydrolysate and 67% sucrose.

Mitosis was studied in squash preparations of pupae and 24 hour old male adult neuroblasts according to Radu <u>et al.</u>,(1975). The medflies were dissected in 1% sodium citrate and the tissues immediately transferred on to a microscope slide with a drop of aceto-orcein. Staining continued for 5-10 minutes, after which observations were made under a microscope.

Polytene chromosomes were obtained from larvae at various stages of the third instar. The larvae were dissected in saline solution (0.7% NaCl, 1 mM Tris pH 7) according to Bedo (1986). Salivary glands were isolated and cleared as much as possible and

then fixed for 5 minutes in a well of a depression slide containing freshly prepared acetic ethanol (1 part glacial acetic acid and 3 parts ethanol) with a change of fixative every 1 minute. Following fixation, the tissues were transferred to a microscope slide and a drop of 2% aceto-orcein added. After 5 minutes of staining the tissue was macerated using a dissecting needle. Tissues were then examined under a low power microscope. When polytene chromosomes were present, gentle tapping and squashing followed to separate and spread the chromosomes which were then observed under a high power microscope.

CHAPTER 3

3.0 RESULTS

3.1 FIELD COLLECTIONS

In all the localities surveyed for the presence of *C. capitata*, it was only completely absent in coffee samples from Kisii district. Other areas showed varying levels of infestation by the medfly as well as differing fruit fly species composition. After picking coffee, the earliest pupae were observed after two days. Upto the tenth day, larvae were still present. Normally larvae pupated from the eleventh to the seventeenth day.

Tables 1-8 show the number of fruit flies obtained from 1 kg samples per locality, mean number of flies per sample, and the species composition in percentages.

C. capitata was the most dominant fly in Kabete and Machakos but occurred in less numbers in Embu and Muranga. In Taita, Kakamega, and Bungoma, production of *C. capitata* from coffee samples was least. *C. nigra* was observed to be the most dominant fly type in Kakamega and Bungoma while *C. rosa* was the most frequent fly in Embu, Muranga and Taita.

Areas west of the Rift Valley, besides having low numbers of *C. capitata*, also showed a markedly low level of fruit fly infestation compared to the areas east of the Rift Valley. Except for Machakos and Kabete which were sampled over a longer period, samples from other areas were collected only once or twice during

Table 1

Ceratitid fruitfly infestation of 1 kg coffee samples from Kabete harvested in January 1988.

Sample No.	*C. capitata*	*C. rosa*	*C. nigra*	Total
1	75	49	35	159
2	26	25	16	67
3	56	32	35	123
4	72	43	29	144
5	36	26	40	102
6	51	47	28	126
7	48	37	19	104
8	59	36	24	119
9	64	51	38	153
10	33	46	46	125
11	46	58	34	138
12	64	49	40	153
TOTAL	630	499	384	1513
MEAN ± S.D.	52.5 ± 15.4	41.6 ± 10.4	32 ± 9.0	126 ± 26.3
PERCENTAGE (%)	41.6	32.9	25.5	100

Table 2

Ceratitid fruitfly infestation of 1 kg coffee samples from Machakos harvested in March 1988.

Sample No.	*C. capitata*	*C. rosa*	*C. nigra*	Total
1	124	57	24	205
2	76	26	14	116
3	256	56	35	347
4	203	48	21	272
5	94	52	29	175
6	149	74	40	263
7	167	31	38	236
8	152	48	26	226
9	98	29	20	147
10	180	73	17	270
11	121	64	23	208
12	86	49	24	159
TOTAL	1706	607	311	2624
MEAN ± S.D.	142.16 ± 53.5	50.58 ± 15.9	25.91 ± 8.2	218.66 ± 64.4
PERCENTAGE(%)	65	23.13	11.87	100

Table 3

Ceratitid fruitfly infestation of 1 kg coffee samples from Embu harvested in June 1988.

Sample No.	*C. capitata*	*C. rosa*	*C. nigra*	Total
1	2	15	-	17
2	8	12	-	20
3	6	7	-	13
4	7	11	-	18
5	12	10	-	22
6	17	13	-	30
7	6	19	-	25
8	4	21	-	25
TOTAL	62	108	-	170
MEAN ± S.D.	7.75 ± 4.7	13.5 ± 4.7	-	21.25 ± 5.4
PERCENTAGE(%)	36.4	63.6	-	100

Table 4

Ceratitid fruitfly infestation of 1 kg coffee samples from Muranga harvested in June 1988.

Sample No.	*C. capitata*	*C. rosa*	*C. nigra*	Total
1	6	47	9	62
2	14	51	10	75
3	4	38	11	53
4	8	61	6	75
5	8	56	5	69
6	5	34	5	44
7	14	68	3	85
8	10	27	8	45
9	11	27	6	44
TOTAL	81	409	63	552
MEAN ± S.D.	9 ± 3.7	45.4 ± 14.8	7 ± 2.6	61.4 ± 15.3
PERCENTAGE(%)	14.5	74.1	11.39	100

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Table 5

Ceratitid fruitfly infestation of 1 kg coffee samples from Taita harvested in January 1988.

Sample No.	*C. capitata*	*C. rosa*	*C. nigra*	Total
1	-	61	6	67
2	1	55	11	67
3	-	39	3	41
4	4	14	1	19
5	2	56	2	60
6	1	24	35	60
7	-	29	9	38
8	-	53	9	62
9	-	38	29	67
10	2	46	18	66
11	-	57	24	81
12	3	21	6	30
13	-	31	11	42
14	1	39	15	55
15	-	24	19	43
TOTAL	14	587	198	799
MEAN ± S.D.	0.93 ± 1.3	39.13 ± 15	13.2 ± 10.1	53.2 ± 16.9
PERCENTAGE(%)	1.75	73.46	24.79	100

Table 6

Ceratitid fruitfly infestation of 1 kg coffee samples from Kisii harvested in November 1987.

Sample No.	*C. capitata*	*C. rosa*	*C. nigra*	Total
1	-	20	16	36
2	-	35	6	41
3	-	8	11	19
4	-	13	14	27
5	-	27	6	33
6	-	15	47	62
7	-	22	31	53
8	-	9	12	21
9	-	13	19	32
10	-	28	43	71
11	-	16	29	45
12	-	19	38	57
13	-	27	27	54
14	-	11	36	47
15	-	20	11	31
16	-	11	13	24
17	-	17	18	35
TOTAL	0	311	377	689
MEAN± S.D.	0	18.29 ± 7.6	22.2 ± 13.0	39.3 ± 15.2
PERCENTAGE(%)	0	45.3	54.7	100

Table 7

Ceratitid fruitfly infestation of 1 kg coffee samples from Bungoma harvested in December 1987.

Sample No.	*C. capitata*	*C. rosa*	*C. nigra*	Total
1	4	25	24	53
2	-	24	33	57
3	1	21	21	43
4	3	22	17	42
5	2	25	44	71
6	1	30	50	81
7	1	11	4	16
8	3	24	23	50
9	-	16	40	56
10	-	21	16	37
11	-	36	11	47
12	2	19	27	48
13	5	18	34	57
14	4	33	38	75
15	1	28	42	71
TOTAL	27	353	424	804
MEAN ± S.D.	1.8 ± 1.7	23.5 ± 6.5	28.26 ± 13.2	53.6 ± 16.0
PERCENTAGE(%)	3.3	43.9	52.8	100

Table 8

Ceratitid fruitfly infestation of 1 kg coffee samples from Kakamega harvested in January 1988.

Sample No.	C. capitata	C. rosa	C. nigra	Total
1	2	18	22	42
2	-	16	17	33
3	-	1	8	9
4	1	8	22	31
5	-	12	16	28
6	-	-	22	22
7	1	8	31	40
8	2	21	16	39
9	4	6	11	21
10	-	11	17	28
11	-	14	21	35
12	-	24	28	52
13	-	30	31	61
14	-	16	12	28
15	2	9	8	19
TOTAL	12	194	282	488
MEAN ± S.D.	0.8 ± 1.2	12.9 ± 8.24	18.8 ± 7.46	32.5 ± 13.1
PERCENTAGE(%)	2.45	39.75	57.8	100

the entire study.

The most destructive stage during the life cycle of C. capitata is the feeding larval stage which is passed in the coffee berries. The larvae do not get into the beans but feed on the soft pulp covering the berries. In edible fruits, this stage is very unpleasant to a consumer. As many as six larvae were recovered in a single berry while most berries had two or three pupae.

In Kabete and Machakos breeding was noted to occur for most part of the year with most flies available during peak coffee ripening seasons.

C. rosa is a bigger fly than C. capitata and both differ in thorax colour, wings, eyes and abdomen colours. C. rosa has white and black crossbands on the abdomen whereas C capitata has yellow and black crossbands. Wings of C. rosa have grey bands and those of C. capitata have black, brown and ochraceous markings. The eyes of C. rosa are green whereas those of C. capitata are wine coloured. C. nigra is black in colour and is easily distinguished from the other two flies.

3.2 LABORATORY COLONIZATION

The medflies reared in an insectary at 22°-25°C and fed on a 3:1 sucrose, yeast hydrolysate medium took between 23 and 28 days to complete their life cycle. Among the fruits offered as media for oviposition, loquat, pawpaw and banana were noted to be the most suitable. The pawpaw medium was however very susceptible to fungal infections which resulted in egg mortality.

At a density of 50 flies per cage, most flies (about 70%) lived for about 60 days after emergence. The longest lived fly was 119 days old and males generally lived longer than females. 30% of the flies survived beyond 60 days. Of those, 83% were males.

The earliest larvae were observed after 6 days following oviposition on fruit media, and the earliest pupae were observed after 14 days. The fruit flies which emerged from coffee were small in size compared to their progeny, although they generally lived longer.

The numbers of F_1 were always low, even when many parents were used to start a line. Often it was impossible to continue with crosses of a lineage due to few parents being available after F_1 generation.

Table 9 shows the origin of flies used to start lineages, duration taken before adults emerged and number of offspring obtained from each cross.

During crosses involving flies from diferrent sampling sites which were done in reciprocals, no eggs were observed oviposited in fruit media or onto a larval medium through oviposition netting material. No progeny was therefore obtained from such crosses. Only flies from Kabete were reared to a second generation. A banana medium was used throughout this cross and adult flies emerged after 23 and 28 days during the first and second generations respectively.

Efforts to have eggs oviposited onto a larval medium through a variety of oviposition netting materials were not successful.

Table 9

Fruitfly generation using fruit oviposition media.

Site	date m/yr		parents M	parents F	F_1 M	F_1 F	F_2 M	F_2 F	l.c.t (days)
Kabete	10/87	loquat	10	10	4	3	-	-	25
Kabete	9/87	orange	10	10	-	-	-	-	
Kabete	10/87	loquat	10	10	7	5	-	-	24
Machakos	4/88	pear	10	10	-	-	-	-	
Machakos	5/88	pawpaw	20	20	10	12	-	-	24
Machakos	5/88	mango	15	15	-	-	-	-	
Kabete	9/88	banana	10	10	12	9	4	5	23, 28
Kabete	10/88	pawpaw	10	10	8	7	-	-	23

F_1 = first generation

F_2 = second generation

M = males

F = females

l.c.t = life cycle time

[illegible] GENETIC VARIATION

[illegible] CHROMOSOMAL OBSERVATIONS

Polytene chromosomes of C. capitata obtained from salivary glands did not fully spread out. The squash preparations showed high degrees of endoreduplication and attempts to spread them out further led to breakages and fragmentation. Weak points and constrictions were visible on the chromosomes (plates 1, 2 and 3) and conceivably fragmentation and breaks occurred at those points.

The polytene chromosomes had a clear banding pattern (Plates 1 and 2). In cytological preparations of C. capitata, polytene chromosomes lack a chromocentre and the centromere is recognised as a diffuse area adjacent to a heterochromatic region (Zacharopoulou, 1986).

Mitotic chromosomes from brain ganglia of one day old adult males showed a distinct six pairs of chromosomes (Plates 4 and 5). The chromosomes were paired to form chromatids which were small in size, highly condensed and in intimate association.
The chromosomes differed in length but due to their small sizes, the different chromosomes were not visualised. The condensed chromatids are apparently in the first meiotic division Due to the chromosome size, only a few characteristics of mitotic chromosomes were discernible. Relaxation of the pairing using colchicine is known to ease observations during chromosomal studies (Radu et al., 1975).

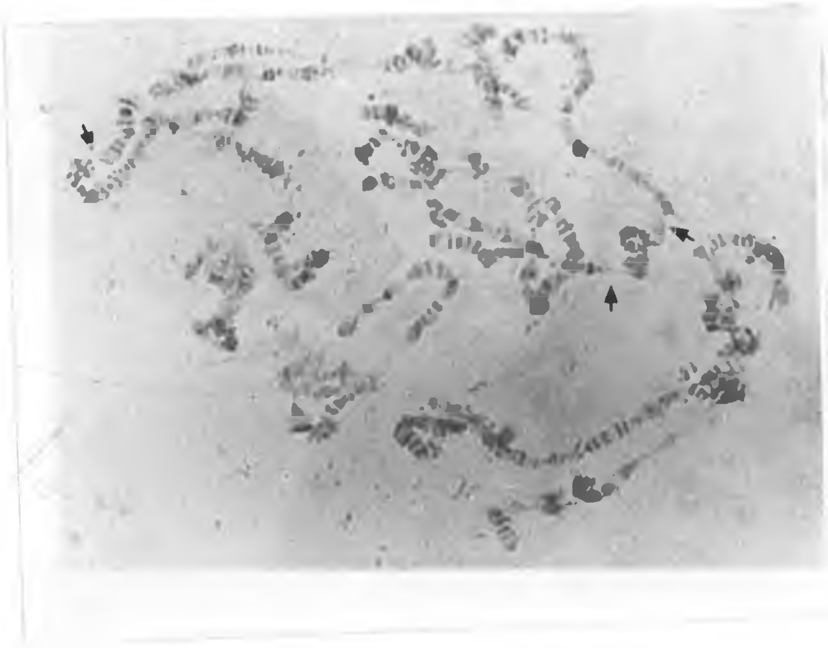

Plate 1

C. capitata salivary gland polytene chromosomes. Arrows indicate constrictions and weak points. X 600

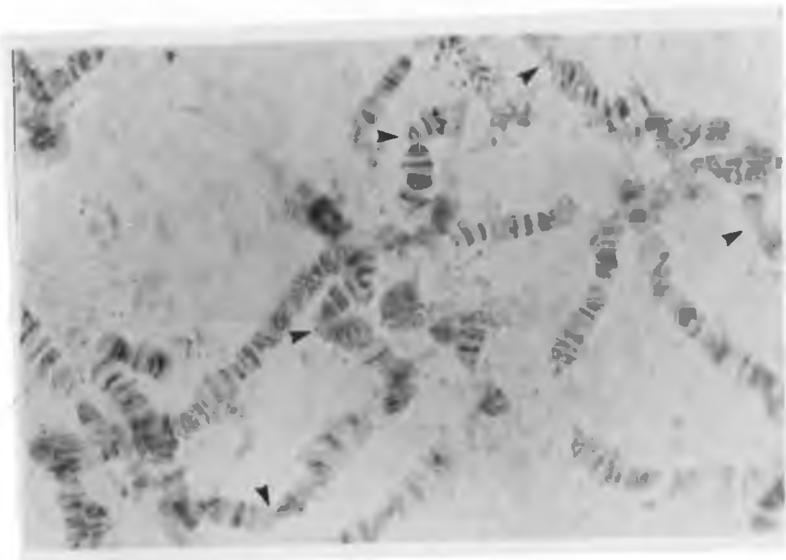

Plate 2

Highly magnified _C. capitata_ polytene chromosomes. Arrows indicate weak points and constrictions. X 1000

Plate 3

C. capitata salivary gland chromosomes. Arrows indicate constrictions and weak points. X 600

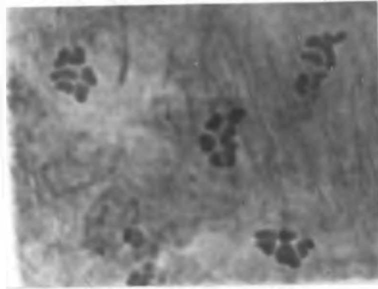

Plate 4

C. capitata brain ganglia chromosomes.
X 1250

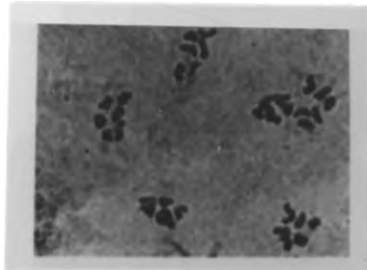

Plate 5

C. capitata brain ganglia chromosomes.
X 1000

3.3.2 ENZYME VARIATION

Tables 10-15 show the allele frequencies for glutamate oxaloacetate transaminase (GOT), β-hydroxyacid dehydrogenase (β-HAD), malate dehydrogenase (MDH), aldehyde oxidase (AO), alcohol dehydrogenase (ADH) and α-glycerophosphate dehydrogenase (α-GPDH).

The β-Had locus had the greatest variation among all loci assayed. The locus exhibited a total of 5 alleles whose phenotypes were either 3 band heterozygotes or single band homozygotes. The bands were detected anodally between 5.6 cm ($\beta\text{-Had}^{86}$) and 10 cm ($\beta\text{-Had}^{145}$) from the origin.

The bands which were thin and wide resolved well on the acrylamide gel and were a bluish colour. Bands appeared in 10 minutes after staining and were fully developed in one hour. The most frequent allele was $\beta\text{-Had}^{100}$ (genotype 100/100) in all localities. Frequencies of other alleles differed from locality to locality. Allele $\beta\text{-Had}^{115}$ was the second most common in Kabete and Machakos. $\beta\text{-Had}^{86}$ was the second commonest allele in all other areas. In flies from Taita and Kabete, 4 alleles were detected whilst only three alleles, $\beta\text{-Had}^{86}$, $\beta\text{-Had}^{100}$ and $\beta\text{-Had}^{115}$ were detected in Bungoma and Kakamega. All the five alleles were detected in flies from Machakos, Embu and Muranga.

The Got locus was observed to have two alleles (Got^{100} and Got^{106}) and was characterised by poor electrophoretic resolution. The Fast Blue RR salt stained the gel very dark and made recording and scoring difficult. Dark blue

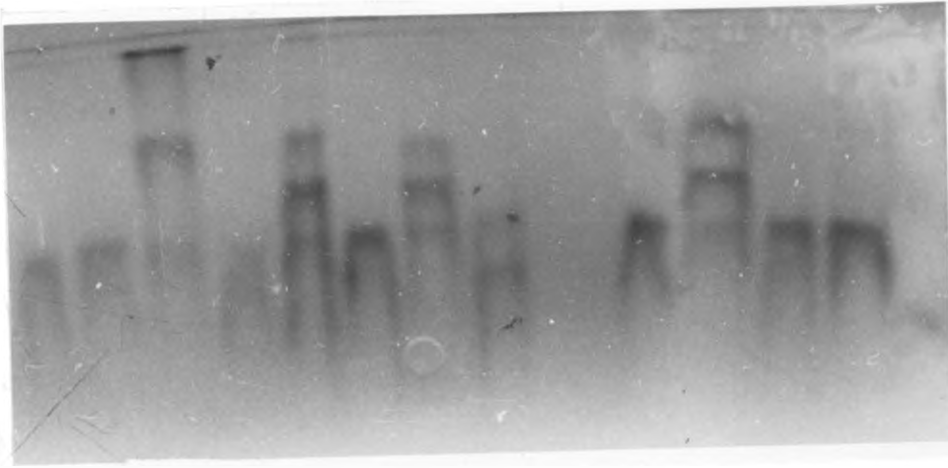

Plate 6

β-Hydroxyacid dehydrogenase (β-HAD) enzyme electrophoretic patterns.

Table 10

β-Hydroxy acid dehydrogenase (β-HAD) allele frequencies

Locality	allele					n
	86	100	115	130	145	
Kabete	0.013	0.732	0.242	0.013	-	75
Machakos	0.037	0.788	0.124	0.018	0.032	78
Embu	0.35	0.574	0.053	0.013	0.01	37
Muranga	0.213	0.573	0.169	0.009	0.036	78
Taita	0.370	0.518	0.074	0.038	-	14
Bungoma	0.415	0.500	0.083	-	-	16
Kakamega	0.375	0.500	0.125	-	-	8

Table 11

Glutamate oxalo acetate (GOT) allele frequencies

Locality	allele		n
	100	106	
Kabete	0.995	0.005	117
Machakos	0.969	0.031	65
Embu	0.917	0.083	39

large bands appeared in 30 minutes and took two hours to full development.

Allele Got^{100} (genotype 100/100) was the most frequent one in all the sampled areas and occurred in more than 90% of the individuals tested. Only homozygotes (genotypes 100/100 and 106/106) were observed at this locus. The fast allele was observed 4.6 cm and the slow one at 4.0 cm anodally from the origin.

The aldehyde oxidase enzyme system was assayed in flies from Kabete and Machakos. The bands at this locus were diffuse, large and streaky and migrated 2-5 cm from the origin (Plate 8). After staining, bands appeared in 10 minutes and were fully developed in 30 minutes.

The allele $\underline{Ao}^{100}$ was the most frequent of the two alleles detected at this locus and was present in 99% and 98% of flies tested from Kabete and Machakos respectively. The most common genotype was 100/100. No 110/110 genotype was observed but genotype 100/110 was present.

Only one allele was detected at the Mdh locus in all the 4 sampled populations indicating that this locus was monomorphic. The MDH enzyme bands were clear and well defined although they varied in staining intensities. At this locus, bands stained a dark blue colour, appeared almost immediately on staining and were best developed after 20 minutes. They however spoiled soon after.

For two populations, only a few insects were sampled. Over 50 insects were however assayed at each of the other sites and all revealed a single allele which migrated 7 cm anodally from the origin.

Plate 7

Aldehyde oxidase (AO) enzyme electrophoretic patterns.

Table 12

Aldehyde oxidase (AO) allele frequencies

Locality	allele		n
	100	110	
Kabete	0.998	0.002	85
Machakos	0.989	0.011	65

Table 13

Malate dehydrogenase (MDH) allele frequencies

Locality	allele	n
	100	
Kabete	1.0	50
Machakos	1.0	65
Kakamega	1.0	7
Embu	1.0	15

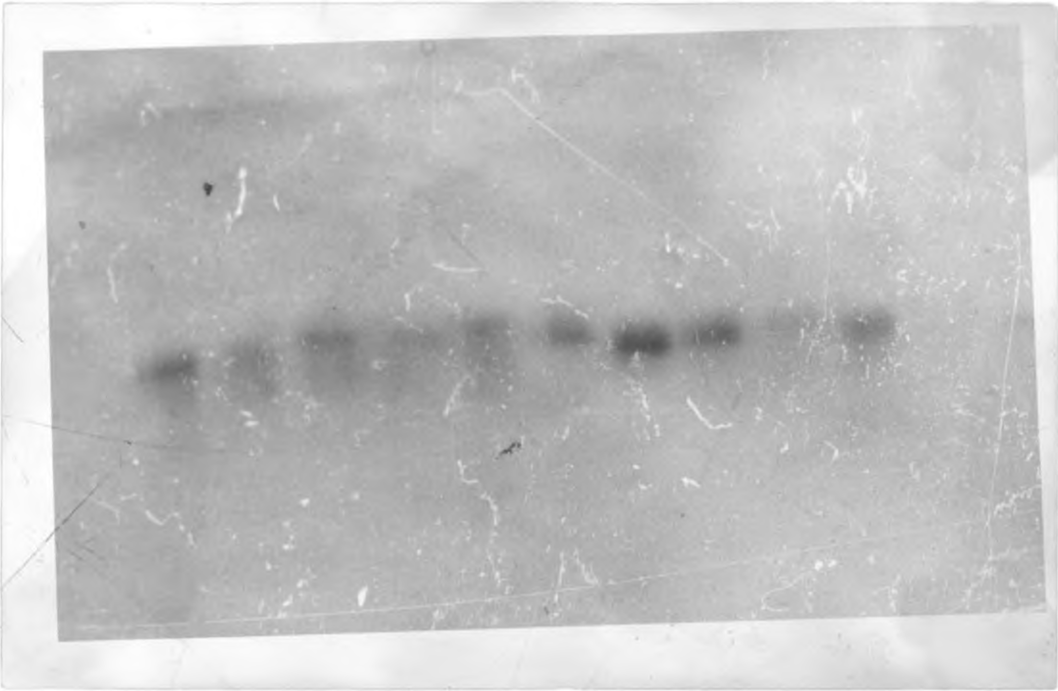

Plate 8

Malate dehydrogenase (MDH) enzyme electrophoretic patterns.

At the Adh locus, two alleles were detected. A slow allele (Adh^{100}) was the frequent one and migrated 5.5 cm from the origin towards the anode. Allele Adh^{110} was detected at 6.5cm from the origin. Bands at this locus were faintly stained although well defined. Bands appeared in 30 minutes and were best developed in one hour.

The genotype 100/100 was the most common followed by 100/110. Few fast allele (Adh^{110}) homozygotes were observed at this locus. The slow allele (Adh^{100}) was observed present in over 95% of the medflies sampled. In two localities, Kakamega and Bungoma, only the commonest allele Adh^{100} was detected. This however may be due to the few insects sampled from the two areas (Table 14).

The phenotypes at this locus were either single band homozygotes or three band heterozygotes. Single band phenotypes were present for both the fast and the slow allele.

The α-Gpdh locus showed little variation both between and within populations. Two alleles were detected at this locus. Flies from Kabete and Machakos displayed both alleles; a fast $\alpha\text{-}Gpdh^{110}$ and a slow $\alpha\text{-}Gpdh^{100}$. From all other locations, only the slow allele was detected. Allele $\alpha\text{-}Gpdh^{100}$ was the commonest. Few heterozygotes were observed at this enzyme locus as three band phenotypes.

Bands developed after 20 minutes and took one hour to full development. The bands appeared between 5 and 6 cm from the origin and stained a pinkish blue. The bands were rather streaky and poorly resolved and showed varying intensities of staining.

Table 14

Alcohol dehydrogenase (ADH) allele frequencies

locality	allele		n
	100	109	
Kabete	0.951	0.049	65
Machakos	0.965	0.035	64
Kakamega	1.0	0	8
Bungoma	1.0	0	6

Table 15

α-Glycerophosphate dehydrogenase (α-GPDH) allele frequencies

Locality	allele		n
	100	110	
Kabete	0.969	0.031	57
Machakos	0.959	0.041	78
Embu	1.0	0	33
Muranga	1.0	0	39
Taita	1.0	0	12
Bungoma	1.0	0	10

6.5 cm

5.5 cm

1 2 3 4 5 6 7

bands

Figure 2

Alcohol dehydrogenase (ADH) electrophoretic patterns. Slow alleles (s) (1 and 2), fast alleles (f) (5 and 7) and heterozygote (3) phenotypes are shown.

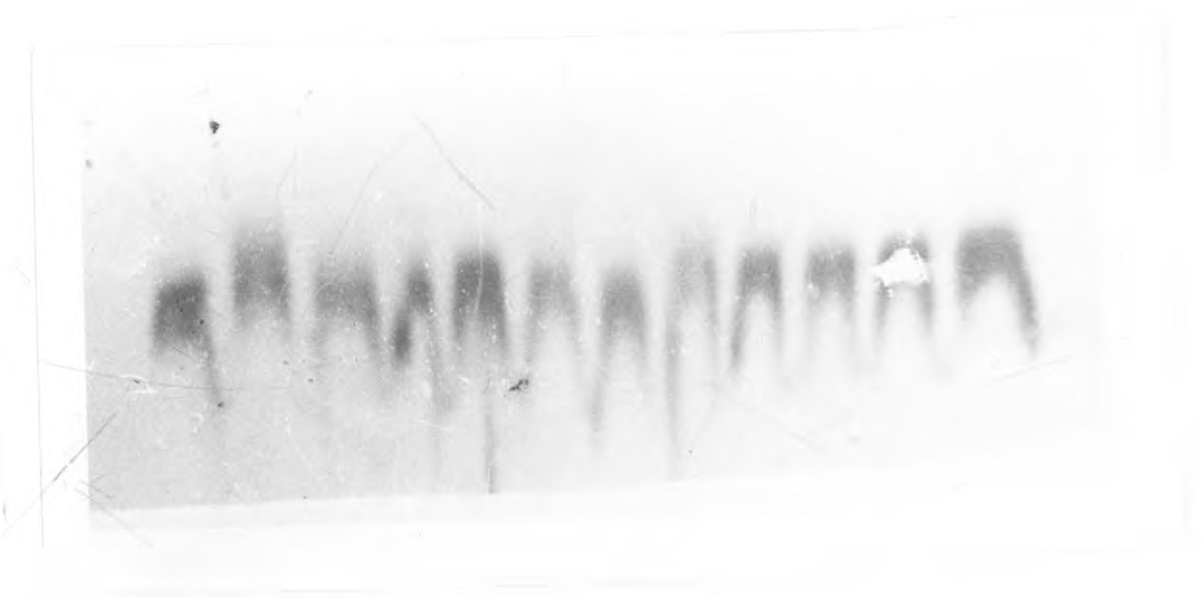

Plate 9

α-Glycerophosphate dehydrogenase (α-GPDH) enzyme electrophoretic patterns

Table 16

Heterozygosity per locus at six enzyme loci

Population	Locus					mean
	β-Had	α-Gpdh	Adh	Got	Ao	
Kabete	0.405	0.075	0.093	0.009	0.003	0.118
Machakos	0.362	0.078	0.067	0.06	0.021	0.117
Embu	0.666	0	-	0.148	0	0.271
Muranga	0.596	0	-	-	-	0.298
Kakamega	0.587	-	0	-	-	0.296
Bungoma	0.569	0	0	-	-	0.189
Taita	0.593	0	0	-	-	0.197
					average mean	0.212

Table 17

Genetic similarities and identities of four population samples. Sampling sites without adequate data are omitted.

	Kabete	Machakos	Embu	Muranga
Kabete	(0.765)			
Machakos	0.996	(0.779)		
Embu	0.945	0.954	(0.723)	
Muranga	0.98	0.977	0.944	(0.698)

I = identity is along the diagonal and

D = genetic similarity is below the diagonal.

Table 18

Chi-square (X^2) values of goodness-of-fit between observed and expected genotypic frequencies under Hardy Weinberg predictions (P = 0.005).

* = significant deviations.

Locus	Locality	n	X^2	d.f.
β-Had	Kabete	75	8.05*	3
	Machakos	78	4.02	2
	Embu	37	10.13*	3
	Muranga	78	20.16*	4
	Taita	14		
	Bungoma	16		
	Kakamega	8		
α-Gpdh	Kabete	57	7.38	1
	Machakos	78	1.899	1
	Embu	33		
	Muranga	39		
	Taita	12		
	Bungoma	10		
Adh	Kabete	65	0.02	1
	Machakos	64	0.34	1
	Kakamega	8		
	Bungoma	6		
Got	Kabete	117	0.02	1
	Machakos	65	0.34	1
	Embu	39	1.98	1
Ao	Kabete	85	1.28	1
	Machakos	65	0.02	1

4.0 DISCUSSION

4.1 OCCURRENCE

In this study, the Mediterranean fruit fly C. capitata was noted to be present in Machakos, Kiambu, Embu, Muranga, Bungoma, Kakamega and Taita districts in Kenya. Waikwa (1979) has reported on the occurrence of the medfly in areas around Nairobi.

The medfly is widely distributed in Kenya's agriculturally productive lands both east and west of the Rift Valley. It is a most dominant fly in Machakos and Kabete while other Ceratitid flies take precedence in Murang'a, Taita and Embu districts. Wherever present, C. capitata was sympatric with C. rosa and C. nigra although infestation levels of the three fruit flies varied from one locality to another.

A worthwhile observation is the absence of C. capitata in coffee samples from Kisii district. Graham (1959) noted C. capitata present throughout the year in coffee and peach plantations. Absence of C. capitata in coffee could be due to non-occurrence or competitive displacement since Mukiama (1985) observed C. capitata in guava samples from Kisii. Fruitflies are known to leave a highly preferred host to a competitor and infest less preferred opes (Debach, 1966). Chemical attractants can be used in such cases to intensify the survey. C. rosa is known to drive out C. capitata in competative displacement, to higher and cooler areas (Myburgh, 1960) and so does Dacus oleae (Debach, 1966).

There is a marked decline of C. capitata numbers from

central Kenya towards western Kenya. Greathead (1972) indicated that T. coffeae (= C. nigra) is the dominant fly type in robusta coffee in eastern Uganda. This trend towards high incidence of C. nigra is well exhibited by samples from Kakamega and Bungoma districts which boarder eastern Uganda.

C. capitata cannot possibly be a serious pest in Kakamega, Bungoma and Taita considering the few insects collected from the areas. The rare occurrence of C. rosa and C. nigra in most parts of central Kenya suggest they may not have adapted to breeding in coffee or may have other preferred ovipositing materials, since many fruits are grown in central Kenya.

C. capitata was the dominant fly in the arabica coffee growing areas and was present in especially high numbers in Machakos. C. rosa was present in all areas sampled and generally occurred in large numbers.

4.2 REARING

It is evident in the results (Table 9), that long term laboratory colonization was not possible. Only from one sample source, (Kabete) was rearing to a second generation possible using banana fruit medium. Other samples and fruit media yielded one generation of fruit flies at most. Orange, Kei apple and mango fruit media did not yield any fruit flies.

While C. capitata has been successfully colonised in laboratories in several parts of the world, difficulties with wild populations have always been present. Heemert and Brink (1980)

reported serious problems in colonising wild flies. Critical factors for mating of medflies are known to exist. For newly captured wild medflies, females are known to be attracted to fruit volatiles, colour, form, fruit size and surface structure. It has been shown that fruit size is a more important factor in acceptance as ovipositing medium than its taxonomic group (Prokopy et al., 1984). Laboratory populations have been shown to accept all materials offered for oviposition equally in a time as short as 5 minutes. Certain light intensities are known to intensify oviposition (Prokopy, 1967b).

Leppla et al., (1983) suggests that the media used in rearing larvae affects fecundity of medflies. Medflies fed on persimons, figs and cactus are more fecund than those fed on artificial media. Medflies were also observed to take about 5 generations before a steady colony was established.

Possible reasons for failure to colonize medflies in the laboratory were, transfer from wild conditions to laboratory conditions, suitability and efficiency of oviposition media and non-fulfilment of critical factors.

4.3 CHROMOSOMAL STATUS

Polytene chromosomes were present in the salivary glands of C. capitata larvae. Radu et al.,(1975) noted polytene chromosomes present in malphigian tubules, midgut, fat body and trichogen cells of C. capitata. The polytene chromosomes displayed a clear well defined banding pattern. Conceivably, several distinct regions which constitute important

landmarks can be identified and thus permit recognition and identification of the various chromosomes. Weak points and constrictions were present in chromosomal preparations of local strains of C. capitata. A high degree of endoreduplication was also observed. Bedo (1986) similarly observed a high degree of pairing in polytene chromosomes of salivary glands but not in those of trichogen cells.

Mitotic chromosomes of brain ganglia were small and highly condensed in 24 hour old males. Counting revealed six pairs which indicate a full compliment of 12 homologues. Local strains of C. capitata therefore have six pairs of chromosomes. Other workers have shown the medfly to have six chromosome pairs with the male being heterogametic with an XY compliment pair (Radu et al.,1975; Southern, 1976; Bedo, 1986). The condensed state of mitotic chromosomes was also observed.

4.4 ENZYME VARIATION

The purpose of this study was among others, to investigate the electrophoretic variation at six enzyme loci and thus determine genetic diversity in several wild populations of C. capitata.

The enzyme -HAD was the most varied and a total of 5 alleles were observed at this locus. Studies by Mukiama (1985) revealed 4 electromorphs and a null allele at this locus in flies from Meru and Kabete. Three populations, Machakos, Embu and Muranga displayed all 5 alleles. Another two populations, Bungoma and Kakamega had only 3 alleles while two populations had 4

alleles displayed. Allele β-Had^{100} occurred in high frequency always. Allele β-Had^{145} was rare in all the localities where present as well as allele β-Had^{130}. In Kabete, the rare allele was β-Had^{86}.

Kakamega and Bungoma populations displayed 3 alleles only and generally lacked rare alleles. The flies from these areas may form a small and isolated population. Machakos, Embu and Muranga, rather adjacent areas show all 5 alleles suggesting they might form a large population together with Kabete where lack of one allele could be due to sampling error. Four allozyme variants have been reported at this locus in Anopheles arabiensis (Mukiama, 1987).

At the GOT enzyme locus, two alleles were detected, Got^{100} and Got^{106}. They were only detected as homozygotes with an obvious shortage of heterozygotes. Allele Got^{100} was detected in 99.5% of all individuals tested, and all sampled sites showed a similar allele frequency pattern. Similar results have been obtained (Kourti et al., 1985) on medflies from southern Africa and Reunion with 2 detectable alleles, one being rare. Ayala et al., (1974) detected between 2 and 6 alleles in different populations of Drosophila species, with a single allele being frequent in all the populations and all the other alleles in low frequencies.

The aldehyde oxidase (AO) enzyme had two alleles electrophoretically detected. The fast allele Ao^{110} was generally a rare allele (Table 13). Fast allele homozygotes and genotype 100/110 were present. In other studies on Kenyan

medflies (Gasperi et al., 1986), this locus displayed a single allele which had a faster mobility than of C. rosa and was diagnostic between the two species.

Aldehyde oxidase enzyme gene is known to be turned on during late third instar larval stage in Drosophila (Wright and Shaw, 1969). The AO enzyme gene has been described and mapped onto chromosome 3 in D. melanogaster and D. simulans (Dickinson, 1970). Electrophoretic variants detected in these Drosiphila species included single band homozygotes and two band heterozygotes.

The enzyme alcohol dehydrogenase catalyses conversion of acetaldehyde to ethanol during glycolysis. At this enzyme locus, two electrophoretic variants were detected manifested either as single band homozygotes, or three band heterozygotes. The observations agree with those of Heemert and Brink (1980) who detected two alleles in wild populations, and a single allele in laboratory cultures of C. capitata. The latter are thought to have lost rare alleles due to small cage populations or through drift. Kourti et al. (1985) reported two alleles at this locus in medflies from southern Africa. Gasperi et al. (1986) also detected two alleles at the Adh locus in Kenyan populations of medflies. In Rhagoletis species, (Berlocher, 1980) three alleles have been detected. They had one or three band phenotypes and 7 different mobilities which are used to distinguish nine species.

In Drosophila, the Adh enzyme gene is turned on just before eggs hatch to larvae (Wright and Shaw, 1969). The Adh

locus has been observed having 5 alleles in 5 populations of *Drosophila* (Ayala *et al*., 1972) although individual populations had one or two alleles, of which one occurred in very high frequency.

Rare alleles were present and allele frequency similarities were observed between Kabete and Machakos as well as between Bungoma and Kakamega.

The malate dehydrogenase (MDH) enzyme catalyses conversion of malate to oxaloacetate in Kreb's cycle. The enzyme was noted to be monomorphic in all medflies assayed. All sampled localities displayed a single allele and similar results have been obtained (Kourti *et al*., 1985) in medflies from southern Africa, Reunion and Mediterranean countries.

In different populations of *Drosophila*, (Ayala *et al*., 1972) a total of 5 alleles have been noted at this locus, although most individual populations had 2 alleles of which one was very frequent. Other studies on the *Mdh-2* locus in *Drosophila* (Ayala *et al*., 1974) revealed three alleles and a marked deficiency of heterozygotes.

The enzyme α-glycerophosphate dehydrogenase (α-GPDH) is found in large amounts in thoracic tissues of flight insects. It is an important enzyme in regeneration of NADH, phosphilipid anabolism and energy production. At this locus, two alleles were detected and one, *α-Gpdh*^{109} occurred at very low frequency and was only observed in two populations. Fast allele (*α-Gpdh*^{109}) homozygotes were absent. One *α-Gpdh* allele was detected (Gasperi*et al*.,1986) in medflies from Kenya, while 4

alleles were present in C. rosa, and this allele permitted discrimination between the two sympatric species.

O'Brien (1967) detected two naturally occurring alleles at the α-Gpdh locus and induced 4 null alleles in Drosophila species. Nulls though fertile had low level of NADH linked α-Gpdh and are thought viable through the use of alternative pathways. A single band at this locus has been observed in D. virilis (Sims,1967). Grell (1967) observed two alleles in D. melanogaster, and the alleles had a three band pattern.

The average mean H of the wild Kenyan populations of C. capitata is 0.212 (table), one of the highest recorded in the species so far. A high H value indicates high genetic variation. Gasperi et al. (1986) obtained a heterozygosity value of 0.166. A slighly higher value of 0.186 was reported by Kourti et al.(1985) in Southern Africa populations of C. capitata.

The chief course of high genetic variation in nature is a huge gene pool. The Kenyan populations of C. capitata can therefore be said to exist in huge numbers mix and breed freely to maintain the observed high genetic variation.

Populations which arise from a few founding members have a limited gene pool and correspondingly little diversity. The Kenyan samples are in the vicinity of Southern Africa and tropical western Africa areas thought to be the origins of the fruit fly. The high variation is further displayed in several samples by presence of rare alleles in some loci like aldehyde oxidase (table 12), alcohol dehydrogenase (table 14) and at the α-glycerol

phosphate dehydrogenase (table 15).

In terms of conventional parameters, the average number of alleles is 2.3 and H is 0.212. Both indicate high levels of genetic variation.

Table 17 shows pairwise comparisons of the genetic similarities between four samples. The genetic identities (I) of different samples show close relationships as expected of individuals of one species (Table 17). Populations from Kabete, Machakos and Muranga display very similar genetic identities. In theory, genetic similarity values range from a minimum of 0 to a maximun of 1. Samples having a same alleles in identical frequencies have a similarity value of unity while those with no alleles in common have a similarity value of 0.

For samples in the neighbourhood of each other, if migration is present, then some alleles are shared in common and differentiation is hindered. The reported findings concern differences as detectable by electrophoresis. The similarities of Kabete, Machakos and Muranga samples show a high proportion of genes in common between the three areas and are therefore genetically homogeneous. A common gene pool through founder members and subsequent migration can be assumed to be the reason for the genetic homogeneity. The similarity is also indicative of similar evolutionary differentiation with respect to random drift, mutation, selection and migration. The three locations are spatially close and indeed share some ecological zones.

The high H values also indicate high genetic variation due to maintenance of a huge gene pool by way of a large number of

individuals who breed freely in a population.

The identity values show the uniqueness of a population's proteins relative to its neighbours. The high I values suggest that despite high similarity values, there exists subtle differences between the populations. Indeed, some alleles are only absent in some samples for example, β-Had^{145} being absent in Kabete while present in Machakos and Muranga. The samples also show variation in the allele frequencies at all the assayed enzyme loci. Small I values are known to suggest a large sampling error (Nei, 1971).

Most genotype frequencies fit well into the expected and can be said to be in Hardy-Weinberg Equilibrium (HWE). Under the HWE, gene frequencies remain constant from generation to generation and migration, genetic drift and selection are assumed to be absent and that mating is random. Three samples, namely Kabete, Muranga and Embu (Table 18) were not in HWE for the β-Had locus. The HWE can be offset by selection, migration, mutation and or genetic drift. At the β-Had locus, five alleles were identified. Some occurred in very low frequencies like β-Had^{145} (0.01) for the Embu sample. Grouping of these small frequencies with other alleles could lead to an impression of Hardy-Weinberg disequilibria. Indeed the disequilibria is only in the three samples where β-Had^{145}, the allele with the least frequency was present. The locus can however be further examined for evidence of selection, non-random mating and gene flow; causes which can lead to disequilibria.

An instance of heterozygote deficiency was observed at the

Got locus, (Table 11) in all the areas sampled. In a population where mating is random and there is no selection, heterozygotes are more common than either class of homozygotes. Cases of heterozygote deficiency are usually due to selection against them. The Got^{100} allele was very frequent (0.995 in Kabete and 0.969 in Machakos) suggesting that matings are likely to produce Got^{100} homozygotes at the locus.

Save at the β-Had locus, all the other five loci were in HWE. This state of equilibrium implies that Kenyan medfly populations are in a stable state with respect to mutations, migration, selection and genetic drift. Any one or a combination of the foregoing offsets the HWE and gene frequencies change from generation to generation.

In conclusion, the investigation revealed that,

1. Rare alleles are present in wild Kenyan populations of C. capitata.
2. Genetic variation varies from locus to locus.
3. Generally, the samples show similar amounts and frequencies of genetic variation.
4. That there is high genetic variation in Kenyan medfly populations, there is evidence of gene flow between samples and that huge numbers are likely to constitute the population.

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