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Genetic diversity in banana weevil *Cosmopolites sordidus*, Populations in Banana growing regions of the world

By

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A thesis submitted in fulfillment for the degree of Doctor of Philosophy in Biochemistry at the University of Nairobi.
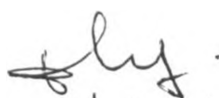
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DECLARATION

I, Ochieng Vincent Oduol, hereby declare that this thesis is my original work and has not been presented for a degree award in any other university.

Ochieng Vincent Oduol

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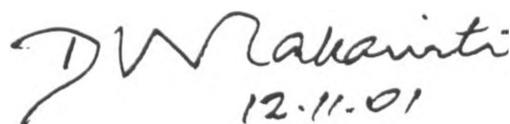ICIPE Supervisor

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DEDICATION

This thesis is dedicated to my wife, Lynette.

ABSTRACT

Genetic variability in banana weevil populations was analyzed in a large number of samples obtained from 15 banana growing tropical countries using random amplified polymorphism DNA (RAPD). A total of 46 RAPD markers were studied. Within polymorphism as measured by percentage of polymorphic RAPDs, varied between 78.3% and 97.8%. Genetic variability was measured using shannon's information index and partitioned into between and within populations components. Overall, genetic variation among *C. sordidus* populations was $(H_{sp}-H_{pop})/ H_{sp} = 25.3\%$. The genetic diversity for the species (H_{sp}) was 83.4%. Mean of within population value (H_{pop}) for all populations was 62.3%. The total genetic diversity was explained by high variation among populations (mean $Gst = 0.213$), which is consistent with low gene flow among populations ($Nm=0.811$). High between population genetic variation observed in this study could be explained by limited migration due to restricted mobility and monophagy in banana weevils.

In a parallel study PCR-RFLP analysis was used to differentiate the CO1 mtDNA of 19 populations of banana weevil. The PCR-RFLP method produced unambiguous profiles that differentiated certain populations from others. In another study, the genetic variability in weevil populations was analyzed in samples obtained from 15 sites in banana growing regions of Uganda. Although the weevil populations in this case were very similar genetically, some variability was observed. The results clearly show genetic diversity in banana weevil.

ACKNOWLEDGEMENTS

gratitude to my wife, Lynette, for the sacrifices she made to enable me complete this study.

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ABBREVIATIONS

Abbreviations	Full Name
ATP	Adenosine Triphosphate
BSA	Bovine serum albumin
bp	Base pair
CTP	Cytosine triphosphate
DNA	Deoxyribonucleic acid
DTE	Dithioerythritol
DTT	Dithiothreitol
EDTA	Ethylenediaminetetra-acetic acid
HA	Hectares
hr	Hour
KB	Kilobase (s)
M	Metre
Mg	Milligram
Min	Minutes
Ml	Millilitre
mM	Millimolar
M	Molar
μ	Micro
μg	Microgram
μl	Microlite
μM	Micromolar

ng	Nanogram (s)
nm	Nanometers
OD	Optical density
Rpm	Revolutions per minute
RNA	Ribonucleic acid
S	Sverdberg unit (s)
SDS	Sodium dodecylsulphate
Sec	Second (s)
Tris	Tris (hydroxy-methyl) aminoethane

CHAPTER ONE

1.0   INTRODUCTION

1.1.   Biology of Banana weevil

The banana weevil (*Cosmopolites sordidus* Germar) evolved in Asia and is now distributed in all major banana-growing regions (Ostmark, 1974; Waterhouse and Norris, 1987). The geographic dispersal of the weevil is not well documented; in most localities, however, the weevil is believed to have been present for at least 100 years. The adult *C. sordidus* live under or in newly cut or rotting pseudostem. The female weevil either lays its eggs in the cut or rotting pseudostems or moves out to the living banana plant where the eggs are inserted single into a hole made by the rostrum. Usually after egg-laying which takes place at night, the female moves back to the rotting pieces of pseudostem. The damage is caused mainly by the larvae, which tunnel extensively into the rhizome and pseudostem (Harris, 1947) and tissues between the lateral roots and the bulb, thus cutting off the flow of sap to the plant (Moznette, 1920). Infested farms show general decline in plant vigour, leading to reduced bunch size (Harris, 1947; Bujulu *et al.*, 1983; Sikora *et al.*, 1989). Infested young plants become stunted and leaves turn yellow and wither, often suckers developing in heavily infested stools are too weak to resist strong winds (Harris, 1947) and easily snap just below the ground level (Bujulu *et al.*, 1983; Sikora *et al.*, 1989).

The effect of climate, or geographical location, does not appear to affect the depredations of this pest. It occurs through the tropics well down into the sub-tropical regions, and is as prevalent and destructive in mountain ranges as on lowlands, both at a distance from and along the seaboard (Froggatt, 1925).

The weevil shows long life span and low fecundity with a classical "k" selected life strategy (Pianka, 1970). Adults are free living (not confined to the banana plant) and may live for two or more years (Froggatt, 1923). Adult weevils cause no significant damage to the banana plant, the function of the adult being purely reproductive. Egg production is low with oviposition estimated from 1 to 2.7 eggs per week (Cuille, 1950; Delattre, 1980; Arleu and Neto, 1984) and 10 to 270 in the lifetime of the insect (Cuille, 1950; Arleu and Neto, 1984; Viswanath, 1977; Castrillon, 1989). After six larval instars, pupation occurs near the surface of the rhizome.

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The duration of the development varies between 23 and 45 days under tropical conditions (Cuille, 1950). After emergence, the adults remain inside their pupal chamber for 6-30 days (Froggatt, 1924) or 22-60 days (Cuille, 1950). Oviposition starts 1-2 weeks after leaving the chamber (Cuille, 1950).

The borer is a blackish brown weevil approximately 9-20 mm long and possesses a short snout from which protrudes a pair of perpendicularly located eight jointed antennae. The elytra are shield shaped with longitudinal lines and terminate above the most distant abdominal segment. Males are generally smaller than females but of similar morphology. Adults of *C. sordidus* inhabit old and decaying plants, infested and decaying leaf stalks, trunks which have been cut and abandoned, and decaying tissue surrounding mats (fig. 1). While weevils show a great attraction for decaying tissue, they move away from tissue which is completely rotten. Some adults have been observed climbing up the vascular tubes at levels as high as four feet above ground. They are extremely sluggish creatures and often feign death both in the field and laboratory. A high degree of clustering behavior is demonstrated (fig. 2) and mating occurs throughout the day (Pinto, 1928).

1.2   History of Banana weevil

In 1824 a black weevil belonging to the group of beetles whose heads are drawn out into a snout or proboscics was described by Germar under the name of *Calandra sordida*, from "India orientali". What eventually proved to be the same insect whose specimens came from Brazil was in 1845 called *Sphenophorus striatus* by Fahraeus. The name was changed to its present form, *Cosmopolites sordidus* by Chevrolat in 1885, who gave as its known distribution Brazil, Java, Ceylon, Malacca, Saigon, China, Reunion (Harris, 1947).
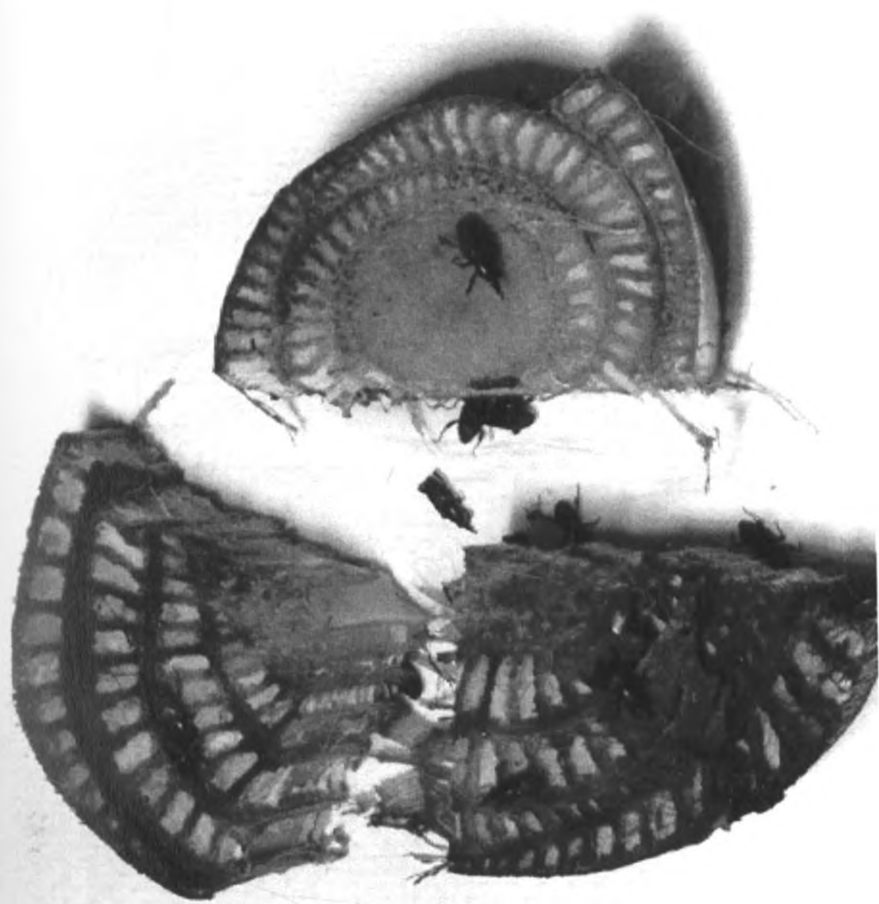
Figure 2:

Banana weevils demonstrating a high degree of clustering behavior in decaying tissue of banana in the field.

It was first mentioned in connection with bananas by Fletraux in 1903 in his account of the beetles of Guadeloupe, as being common in rotting bananas. It was not until 1906 that it was regarded as a pest of bananas, and an account given of its destructive powers on the Island of Sao Tome in the Gulf of Guinea. It appears to have been observed as a pest about the same time in localities as far apart as Madagascar and Fiji, so that there is no indication of where it originated or how it spread, except that it probably moved around the tropics with the edible banana as it was spread by man from its original home in the East Indies (Harris, 1947). Knowles noticed important damage in Fidji in 1908. In 1912 Knowles and Jepson published results on nature of damage and biology of the weevil and suggested that invasion goes back to around 1901 (Knowles and Jepson, 1912). In 1917 Knowles noticed that weevils were spreading all over the Island causing very important damage in several areas (Knowles, 1918).

The weevil was described by Chevrolat in Malasia as early as 1885 (Chevrolat, 1885). In 1915, Saasscer discovered banana weevils in America from banana originating from Phillipines (Sasscer, 1915). Between 1916-1917 Tryon reported the presence of banana weevil in Australia (Tryon, 1917). In 1919 Froggatt found banana weevil in some areas of New Wales (Froggatt, 1922). According to Tryon and Benson banana weevil was introduced to Australia around 1895 with plants originating from Jamaica (Tryon and Benson, 1920). In 1941 Froggatt found low population of banana weevil in New Guinea (Froggatt, 1941). In 1885 Chevrolat reported presence of banana weevil in China and Vietnam (Chevrolat, 1885). Banana weevil has been eshtablished for a long time in the Indies (India, Pakistan, Srilanka) according to Chevrolat. He reported the presence of the weevil in Srilanka in 1885 (Chevrolat, 1885). Moznette reported weevils presence in Florida (North America) in 1913. Since 1920 it has disappeared from the U.S after severe control measures (Moznette, 1920). It was reported in South America in Brazil in 1885 by Chevrolat.

On the African continent it was first observed in the lower Congo in 1913 in the region of Mayombe (Ghesquiere, 1929). It was reported by Gowdey in Uganda in 1918, with his opinion that it was not of recent introduction (Gowdey, 1919). Though not recorded by the Germans in Tanganyika prior to 1914, it was found to be well established at Amani by A. H. Ritchie in 1922.

By 1936, banana weevil had been recorded in most parts of Uganda. Four years later it was found established in the adjoining Bukoba district of Tanganyika and on islands in the extreme south of Lake Victoria (Harris,1943). In 1945 specimens of the pest were identified from Zanzibar and in the following year, from Kilimanjaro area of Northern Tanganyika. In the same year it was stated to be generally distributed in the French West African colonies. Banana weevil existed in Sierrs-Leone as early as 1925 (Hargreaves, 1925). It was reported in South Africa in Natal in 1924 (Anonyme, 1926). A considerable time elapses between the first arrival of *Cosmopolites* in a new area and it becoming a serious pest. It is estimated that this took about ten years in Uganda (Hargreaves, 1940). There is very little information available on the introduction of the banana weevil in Kenya.

1.3. Banana evolution and importance

Bananas originated in South and South-east Asia from two wild progenitors *Musa acuminata* (donor of A genome) and *Musa balbisiana* (donor of B genome), and then spread throughout the humid tropics (Stover and Simmonds, 1987). Secondary centers of crop diversity exist in East Africa (highland cooking bananas, unique to the region) and West Africa (plantains) (Stover and Simmonds, 1987).

Banana and plantains are some of the most important food crops in the world. Grown principally in developing tropical countries, the total annual production of bananas and plantains is 85.5 million tonnes. There are two main types of bananas: dessert bananas and cooking bananas. While dessert bananas are exported to Europe, the United States and Japan (9 million tonnes annually), cooking bananas are an important carbohydrate source in developing countries.

Bananas are an important food crop in many tropical and subtropical regions and are the primary source of dietary carbohydrates, vitamins and minerals for more than 400 million people (McNicoll, 1989). In large parts of East-African highlands, bananas are the primary food source with per capita consumption reaching 220-460 kg per annum (INIBAP, 1987). The countries around the Lake basin, viz. Uganda, Tanzania, Rwanda, Burundi and Kenya alone produce and consume 18 % of the world banana production (FAO, 1991). Most of the produce is consumed within the region and exported quantities are rather low. Initially, banana germplasm probably entered East Africa from two directions: the human inhabited tropics of Western and Central

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Africa, and the Indian Coast. Yet the number of distinctive clones recorded in East Africa suggests the importance of the region as a secondary center of diversity (Davies, 1995).

Edible bananas (*Musa* spp.) comprise a genetically diverse crop encompassing a range of diploids, triploids and tetraploids (Stover and Simmonds, 1987). Triploidy gives various benefits, most importantly triploid plants tend to be more vigorous and productive than diploids. The vast majority of cultivated types are triploid. These now constitute important staple and/or commercial crops in Sub-Saharan Africa, Latin America, Asia, the Pacific and Australia. Banana and plantain production constraints include a pest complex (weevils, nematodes and diseases) which cause serious yield losses and shortens plantation life (INIBAP, 1986; Sebasigari and Stover 1988; Swennen, 1984; Gold *et al.,* 1993). Pest status, in turn, is influenced by genome group. In Africa, for example, the banana weevil, *Cosmopolites sordidus (* Germar*)*, is considered a primary production constraint of highland cooking bananas and plantains but not of dessert or brewing bananas. As such, it has been reported as the major biotic constraint to banana production in Eastern Africa (Persley and de Langhe, 1987) and either the first or second (after black sigatoka disease) most important plantain pest in West Africa (INIBAP, 1988a). Uganda is the world's leading producer and consumer of cooking bananas. In recent years, a decline in production, largely attributed to pest pressure has led to cultivar shifts and replacement by annual food crops e.g. cassava, sweet potatoes and maize in central Uganda (Gold *et al.*, 1993). In central Uganda, farmers have attributed disappearance of highland cooking bananas, in large part, to increased banana weevil problems.

In Kenya, the banana is cultivated on about 74,000 hectares, which makes up 1.7 percent of the country's total land used for crop production (MALDM, 1997). Banana ranks eighth in the Kenya government's crop priority list enlarging food production in order to keep pace with population development (Nguthi, 1996). In all of Kenya's eight provinces bananas are grown, although the four major growing provinces: Nyanza, Central, Eastern and Western make up 90 percent of total national production (Table 1).

The most constraining pests in all banana growing regions of Kenya are banana weevils and nematodes, particularly *Radopholus similis* and *Pratylenchus goodeyi* (Table 2). Host plant resistance for these pests is not known in any of the domesticated or wild banana species, although some varieties show a moderate degree of tolerance to nematodes (Sarah, 1993). Farmers are often faced with gross damage caused by these pests, which can be quite severe.

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Yield reductions due to weevils, for instance can easily reach 50 percent. Losses are increased where there is an interaction between weevils and nematodes (Speijer *et al.*, 1993).

Table 1 : Banana production statistics for the Provinces of Kenya (1996-97 averages)

Province	Area (ha)	Production (t)	Yield (t/ha)	Production share (percent)
Central	16,913	169,316	10.0	16.5
Coast	5,743	55,341	9.6	5.4
Eastern	9,669	97,144	10.0	9.5
Nairobi	48	409	8.5	0.0
North Eastern	271	1,522	5.6	0.1
Nyanza	30,234	574,740	19.0	56.1
Rift Valley	2,688	39,781	14.8	3.9
Western	7,800	86,107	11.0	8.5
Total	**73,366**	**1,024,360**	**14.0**	**100.0**

Source: MALDM (1996,1997).

Table 2. Altitude, banana type, age of banana plants, percent coefficient of infestation (PCI), and numbers of banana weevils and nematodes in banana fields in Kenya, 1989. (Source: Waudo, 1998)

District	Altitude (m)	Banana Type	Age (Years)	PCI for weevil	Pratylenchus Goodeyi/ 100cm³ soil	Pratylenchus Goodeyi/ 1g dry root	Radopholus similis/ 100cm³ soil	Radopholus similis/ 1g dry root	Helicotylenchus ssp. 100cm³ soil
Kakamega	1460	Cooking	6	83.3	30	0	10	0	80
	1460	Sweet	6	40.00	0	0	0	0	0
	1530	Cooking	7	73.33	20	14,286	10	0	0
	1530	Sweet	7	53.33	20	0	0	0	0
Bungoma	1500	Cooking	5	13.33	440	0	0	20	30
	1500	Sweet	5	30.00	20	0	20	0	100
	1650	Cooking	30	3.30	110	125	10	0	30
	1650	Sweet	30	23.30	0	0	0	0	0
Busia	1160	Cooking	15	100.00	0	0	20	0	130
	1160	Sweet	15	86.33	00	0	0	0	340
Siaya	1330	Cooking	11	100.00	0	0	0	0	60
	1330	Sweet	11	76.66	10	0	10	0	30
	1470	Cooking	11	93.33	0	0	0	0	60
		Sweet	11	40.00	0	0	0	0	80
Kisumu	1430	Cooking	5	60.00	190	0	0	0	0
	1430	Sweet	5	10.00	50	0	0	0	0
	1470	Cooking	5	83.33	470	0	0	0	0
	1470	Sweet	5	53.33	0	0	0	0	0
South Nyanza	1330	Cooking	2	100.00	0	11,111	0	0	250
	1330	Sweet	2	36.70	0	0	0	0	0
Kisii	1470	Cooking	4	80.00	120	0	30	0	0
	1470	Sweet	4	33.33	30	0	0	0	20
	1740	Cooking	7	90.00	180	0	0	0	0
	1740	Sweet	7	33.33	60	0	20	0	0
Trans Nzoia	1800	Cooking	15	33.33	70	0	10	0	90
	1800	Sweet	15	13.33	20	0	20	0	70
Kericho	1770	Cooking	9	50.00	20	0	0	0	0
	1770	Sweet	9	20.00	0	10,000	0	0	0
Meru	1940	Cooking	5	6.66	10	80,000	10	0	0
	1940	Sweet	5	0.00	0	50,000	0	25,000	0
	1510	Cooking	11	56.66	10	20,000	0	0	0
	1510	Sweet	11	23.33	10	6,250	0	6,250	0
Machakos	1580	Cooking	14	43.33	10	0	80	33,333	70
	1580	Sweet	14	23.33	10	0	0	0	0
	1650	Cooking	5	6.66	0	0	0	0	0

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Region									
	1650	Sweet	5	6.66	50	63,636	0	0	0
Nairobi	1780	Cooking	6	10.00	30	277,778	0	4,762	20
	1780	Sweet	6	0.00	40	52,174	0	0	10
	1530	Cooking	3	0.00	90	10,110	20	5,304	30
	1530	Sweet	3	0.00	0	0	10	0	0
Nyeri	1700	Cooking	5	20.00	0	4,348	0	0	0
	1700	Sweet	5	13.33	0	0	0	18,519	0
	1580	Cooking	5	0.00	0	10,000	0	90,000	0
	1580	Sweet	5	0.00	20	18,182	0	9,091	10
Embu	1490	Cooking	4	20.00	0	0	10	0	0
	1490	Sweet	4	16.70	10	10	0	0	0
	1470	Cooking	4	96.66	60	37,500	0	25,000	0
	1470	Sweet	4	50.00	0	0	0	0	0
Kirinyaga	1330	Cooking	4	70.00	20	0	10	25,000	0
	1330	Sweet	4	90.00	10	0	0	0	20
	1470	Cooking	5	0.00	0	0	0	0	0
Kiambu	2040	Cooking	7	96.66	0	0	0	0	10
	2040	Sweet	7	10.00	0	0	0	0	0
Muranga	1215	Cooking	5	40.00	0	20,000	0	0	0
	1215	Sweet	5	33.33	0	0	0	0	0
	1190	Cooking	5	46.66	0	0	0	0	0
	1190	Sweet	5	43.33	0	0	0	0	0
Kilifi	10	Cooking	25	26.66	10	0	0	0	200
	10	Sweet	25	10.00	0	0	0	0	200
	20	Cooking	2	83.33	0	11,111	0	0	1,800
	20	Sweet	2	76.66	0	0	0	0	60
Mombasa	40	Cooking	4	10.00	0	0	0	0	140
	40	Sweet	4	13.33	0	50			
	32	Cooking	4	10.00	0	0	0	0	0
	32	Sweet	4	0.00	.50	0	0	0	0
Kwale	125	Cooking	5	32.33	0	0	0	0	60
	125	Sweet	5	36.66	0	0	0	0	460
	210	Cooking	1	96.66	0	0	0	13,333	130
	210	Sweet	1	33.33	0	10	0	0	50
Taita Taveta	800	Cooking	3	23.32	0	0	10	0	60
	600	Sweet	3	100.00	20	12,500	0	0	60
	1420	Cooking	9	43.33	0	7,692	0	0	0
	1420	Sweet	9	50.00	20	9,524	20	0	20

1.4.   Uses of Banana (Musa)

Bananas are the largest herbaceous plant. The fruit is a rich source of carbohydrate (35%), fibre (7%), minerals (potassium, magnesium, phosphorous, calcium and iron) and vitamins (A and C) (Table 3). While dessert bananas are consumed directly after ripening, cooking bananas are boiled, roasted, fried, steamed, baked, brewed or dried and ground into flour (Table 4). Dried Banana plant sheaths are woven and used for thatching, making ropes, cots, bandages, hats, ornaments and as shading. The pseudostem fibre is used for fishing nets, cattle feed and mulch (Mbwana, 1998).

Table 3. Mineral content of banana Leaves [1]

Macronutrients	
Nitrogen*	2.6-4.0%
Phosphorous	0.19-0.25%
Potassium*	2.6-3.0%
Calcium	0.75-1.25%
Magnesium	0.3-0.46%

[1] Values represent normal range of minerals by dry weight.
*Major nutrients.

In 1998, bananas were ranked third most important carbohydrate staple in the world after cassava and sweet potato. In Africa, 24.6 million tonnes of bananas are produced annually, making them the third most valuable crop after cassava and maize. In East Africa, bananas are a staple food for more than 20 million people. The average annual consumption per person in the East African highlands near the Lake Victoria basin is 250-350 kg. In Europe, the annual consumption of dessert bananas per person is 11kg (Mbwana, 1998)

1.4.1. Alcohol Production

In central and East Africa, the juice from the ripe fruit of varieties known as 'beer bananas' is drunk fresh or fermented to make a beer of low alcohol content. Consumption in Rwanda may reach 1.2 litres per capita per day. Bananas are among the crops giving the highest yields in the production of commercial or medical alcohol per hectare.

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1.4.2. Animal Feed

Cattle and pigs relish ripe bananas but the unripe fruit can also be dried and made into a meal which can be used to substitute up to 70-80% of the grain in pig and dairy diets with little change in performance.

1.4.3. Medicinal Use

Because they contain vitamin A, bananas and plantains act as an aid to digestion. There are also reports of the ripe fruit being used in the treatment of asthma and bronchitis. The pounded peels of ripe bananas can be used to make a poultice for wounds and, as the inside of the peel has antiseptic properties it can be wrapped directly around wounds or cuts in an emergency.

1.4.4. Source of Fibre

Bananas and plantains are the source of a fibre used extensively in the manufacture of certain papers, particularly where great strength is required. The paper is used for, among other things, making tea bags and bank notes. The fibre has numerous other uses, including textile manufacture, for making ropes, string and thread, and for the production of various handicrafts.

1.4.5. Other uses

The large leaves of bananas make ideal green umbrellas and they are frequently used as disposable 'biological' plates. They can also be used for thatching, for wrapping food during cooking, as bowl covers and tablecloths, as temporary mats and for the covering of earth ovens to hold in the heat. Starch can be extracted from banana and plantain pseudostems and such starch has been used for producing the glue used in the manufacture of cartons for exporting fresh bananas. In mixed farming systems, bananas are used as a ground shade and nurse-crop for a range of shade-loving crops including cocoa, coffee, black pepper and nutmeg. While the plants themselves are prized as ornamentals worldwide, the seeds from wild species are used for making necklaces and other ornaments. Banana sap can be used as a dye, and banana ash is used in soap manufacture. In Indonesia, the production of floor wax and shoe polish from banana peels is also being explored (IPGRI, 1996).

1.5. Banana Cultivars

A number of different cultivars are available, and the variety selected for growth depends upon environmental conditions and desired end-use. Banana cultivars respond differently to weevil and nematode attack. The sweet dessert types are generally more tolerant, followed by cooking types, beer varieties and the roasting types (Mbwana, 1998).

Originally, there were two wild species of banana plants, *Musa acuminata* and *M. balbisiana*. With time, these two species hybridised to generate the numerous cultivars available today. The genetic *acuminata*-types of bananas (AA) originated in Malaysia and hybridisation between sub-species gave rise to a range of diploid (AA) and triploid (AAA) cultivars. The *balbisiana*-types (BB) originated in the Indian sub-continent. The spread of AA-types through Southeast Asia to the Indian sub-continent led to hybridisation between AA and BB types, which gave rise to the AAB and ABB cultivars that are grown worldwide today.

Bananas with the AA or AAA genomes are typically sweet and are cultivated as dessert bananas. Hybrids with AAB (plantain) and ABB genomes are starchier and therefore used as cooking bananas. AAB and ABB hybrids are grown in coastal lowlands, while the AAA-type grow well at elevations of 1000-1800 metres. It is the AAA-varieties that are widely grown in the East African highlands. These highlands are a system of high plateau, mountains, valleys and lakes, which extend across Ethiopia, Eritrea, Burundi, Kenya, Rwanda, Tanzania, Uganda and the Democratic Republic of Congo. This region of high elevation receives intermittent heavy rainfall and high winds and is often sloping land. The total annual production of banana and plantain in this region is 13.2 million tonnes, about 20% of the global production.

In Kenya a wide range of banana varieties is grown. The first obvious separation between varieties can be made between the ripening (dessert) bananas on the one hand, and the cooking type on the other. Some of the varieties are also used for both purposes. Ambiguity can occur with the term "plantain". For many, plantains represent all cultivars that are used for cooking. Others confine the expression to a subgroup of the AAB triploid hybrids, which constitutes only a small fraction of all commonly used starchy cooking type varieties (Robinson, 1996). There appears to be no generally accepted rule of distinction between bananas and plantains. According to FAO statistics, the Kenyan area cultivated with plantains is more than double the area under bananas (FAO, 1998). Kenyan researchers and national statistics, however, say that the use of plantains in the country is negligible. Here, only the term banana is used, with the

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description differentiating between ripening and cooking type varieties. According to interview survey, about half of the Kenyan banana production is of the ripening type whereas the other half is made up of cultivars mostly used for cooking. Among the ripening varieties there are some, which have been used for a long time in Kenya. The most prominent of which are the Gros Mitchel (AAA), locally often named Kampala, and apple banana (AB). Other ripening types have been introduced to the country more recently, especially those of the Cavendish subgroup (AAA), such as Dwarf Cavendish, Chinese Cavendish, Lacatan, Valery, Grand Nain, Williams, Paz and others, generally with a fairly good acceptance among banana producers and consumers. Many of the cooking varieties used in Kenya belong to the subgroup of East African highland bananas (AAA). Not all of them have been genetically identified, so that some appear with different local names, although in cytological analysis they turn out to be very similar. Local names of popular cooking varieties include Matoke, Kiganda, Mutahato, Bokoboko among others. A commonly used double purpose cultivar is Muraru (AAA) (Qaim, 1999).

In Queensland, the Cavendish, Sugar, Lady's Finger, plantain, Gros Michel and Dacca varieties of banana plants have all been found to suffer from the attacks of the banana weevil borer (Shani,1990). All species of the Genus *Musa* are attacked by the banana borer weevils *Cosmopolites sordidus* GERM and no cultivar is known to possess any useful degree of resistance to the pest (Simmonds, 1966). However, it has been reported that some cultivars are more susceptible to the borer attacks than others. 'Gros Michel' cultivar was reported to be more susceptible than 'Lactan' (Hord and Flippin (1956). In Jamaica 'Valery, 'Lactan' and the tetraploid (*Musa* AAAA) 65-3405-1 were found to be only 'lightly' infested with borers. In Panama 'Valery' and 'Manzano' cultivars were found to be relatively resistant to this pest (Feakin, 1971).

Table 4. East African Highland banana cultivars and their use

Cultivar group	Cultivars	Areas of common Occurrence	Use
Mboko	Mboko Ibwi Muhonye Ntebwa	Usambara Mountains, Tanzania	Cooking
Mshare	Nshonoa Mnanambo Mlelembo	Kilimanjaro & Meru Mt areas	
Matoke	Nakyetengu Mbwairuma Nshakara	Lake Victoria basin, highlands	
Cavendish	Kiguruwe Robusta Paz	Coastal, Lowland areas	
Plantain	Mkono wa Tembo Mzuzu	Coastal, Lowland areas	Roasting
Mbire	Nshanshambire Ntalibwambuzi Enyamawa	Lake Victoria basin, highlands	Brewing
Kisubi	Kisubi or Kijivu	All banana areas	
Kijoge	Gros Michel Or Bogoya	Lake Victoria basin, highlands	Dessert
Kisukari	-	All banana Areas	
Bluggoe	Bokoboko Ngazija	Coastal, lowland areas	Multi-purpose

1.6. Banana Pests and diseases

Bananas are stressed by a number of biotic factors, particularly in the humid tropics where the climate is favourable for the propagation of pests and diseases.

1.6.1. Banana diseases

Leaf spot (Sigatoka) is caused by *(Mycosphaerella musicola)* and the symptoms are small yellow or greenish-yellow streaks, parallel to the leaf veins. Later the streaks darken and expand laterally to form elliptical brown spots. The centre of each spot eventually dries up to form a light- grey structure, like "eyespot" or scorching appearance. It is controlled by using resistant cultivar or spraying with benomyl. The fungal disease of banana black sigatoka caused by *Mycosphaerella fijiensis* was reported in Kenya in 1989 (Kungu *et al.* 1992). It can cause yield losses of up to 50 percent. The fungus is spread by the wind or by water and attacks the leaves of the plant. Black sigatoka has so far only been reported in Coast, Central and Eastern provinces. The disease can be controlled by systemic fungicides, but their use is environmentally harmful and often beyond reach of small holder farmers. Most of the banana varieties commonly used in Kenya are affected by black sigatoka, including the Cavendish types. Yellow sigatoka, (*Mycosphaerella musicola*) does occur but it is much less damaging than the black sigatoka.

Panama disease (*Fusarium* Wilt) is caused by soil-borne fungus *(Fusarium oxysporium)*. This fungal disease is often referred to as Panama disease, since it was in Central America where it caused oppressive problems at the end of the last century. The first symptom is yellowing of older leaves or collapse of the petiole while the leaves are still green. All leaves eventually collapse and die hanging on the pseudostem. Later, pseudostem may split. It is controlled by use of resistant varieties e.g. Lacatan or other Cavendish cultivars are recommended, good sanitation and disease free material. It is the most important disease in Kenya. In Kenya the disease was observed for the first time in 1952, but a severe outbreak has only been reported in more recent years (Kungu, 1995). Today Panama disease endemic is all over the country's banana growing regions. However, the severity varies because of the varying susceptibility of different varieties and different regional varietal preferences. In Coast province, for instance, *Fusarium* is not considered a constraint to the same degree as it is in the other provinces, particularly in Central and Eastern, where the strongly affected varieties Gros Michel (Kampala) is most popular. In other parts of the world Panama disease outbreaks in the 1950's and 1960's led to the complete

replacement of Gros Michel by the resistant varieties mainly from the Cavendish subgroup. Other susceptible varieties grown in Kenya are apple banana, Bokoboko and Muraru. *Fusarium* infects the plant's root system and is spread through unclean sucker material, surface water runoff and infected soil carried around on farm implements. The fungus can completely destroy the whole plantation within a couple of months and even when the host plant itself is removed from the plot it can endure for decades in the soil (Qaim, 1999).

1.6.2. Banana pests

Banana Weevil *(Cosmopolites sordidus)* is a major pest of bananas. The larvae feed and tunnel in the corm of the plant. The leaves of infected plants are dull yellow green and floppy. Young sucker often wither and fail to develop.

Stem borer weevil *(Odoiporus longicollis)* bores into the stem and the leaves of the infected plants turn yellow and the plant becomes susceptible to wind damage.

Nematodes *(Radopholus similis)* are microscopic worm-like organisms which feed on the roots of bananas and infect roots and they turn reddish brown and later become black. The roots become short, blackened and reduced in number and thus, susceptible to wind damage.

Fruit fly *(Bactrocera musae)* larvae feed on the fruit. Evidence of attack is indicated by black spot on the skin.

1.7. Integrated Pest Management (IPM) Strategies in control of banana weevil.

An integrated management program for banana weevil borer will allow banana producers to minimize insecticide treatment, associated costs and environmental problems in the face of insecticide resistance in the pest and worldwide pressure to reduce pesticide usage. The extensive application of pesticides has not only upset the delicate biological equilibrium of nature, but it also poses a danger to human health and causes problems of resistance. As a result there is worldwide shift towards integrated pest management (IPM) to the forefront of pest control.

No single control strategy will be likely to provide complete control for banana weevil according to previous research results. Chemical pesticides, especially dieldrin and other cyclodienes such as aldrin, have been used extensively for the control of banana weevil (Bujulu *et al.*, 1983; Edge, 1974; Whalley, 1957), but widespread resistant to these pesticides has

developed in most parts of the world (Foreman, 1973; Edge, 1974; Bujulu *et al.*, 1983). The weevil has been repeatedly the subject of attempts at classical biological control (Waterhouse and Norris, 1987). The natural enemies mostly beetles found preying upon weevil larvae in decomposing banana tissues, have been used for biological control. They were successfully established in a number of instances, but no significant reductions of the pest were achieved. A major reason for some of these failures might have been that the pest's biology was not taken into consideration sufficiently. Therefore, a broad integrated pest management (IPM) approach might provide the best chance for success in controlling this pest. The components of such a program would include habitat management, biological control, host plant resistance and (in some cases) chemical control. The success of any of these control strategies will need to account for local biotypes and related physiological and behavioral differences. For example, weevil biotypes may influence the efficacy of resistant host plants, biological control agents, biopesticides or semiochemicals.

1.8.   The banana weevil biotypes

The weevils seldom, if ever, fly and dispersal by walking is limited. Gold and Bagabe (1994) found that movement of adult weevils was limited, although some marked individuals moved more than 30 m in five days while, Delattre (1980) recorded a maximum movement of 60 m in five months.

Migration of weevils into banana plantations and movements in the plantation have been studied before (Wallace, 1938; Simmonds, 1929). Measurements made at Kawanda Research Station, Uganda, on movement all support the previous findings that the weevil is very sedentary. Of 400 marked weevils released at one point in the plantation, 35 % were recovered over an eight-month period within a radius of ten yards of the original release point. No evidence of flight has been detected and the only times in the laboratory that it has been possible to make the weevil spread its wings were under conditions of extreme drought or in the latest stages of the influence of insecticide.

Dissemination of banana weevils is therefore, predominantly through infested planting material and other ways. The dispersion of the pest may be brought about by transportation of suckers from an infested area, by crawling from an old infested area, more particularly when the supplies of food and breeding material dwindle; this is more applicable where the areas are

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adjacent, by wash down gullies and steep slopes. Many of the best banana areas are situated well up on the hillsides and under the often torrential downpours of rain experienced, infested plant material and beetles in the soil are carried down from high to low levels (Frogatt, 1925). Restricted mobility and monophagy leads to discrete populations that are confined to banana plantations. Local selection and a buildup of genetically distinct biotypes may occur in these isolated populations. The existence of such biotypes might explain the differences in biology across sites (activity in wet versus dry seasons; oviposition potential; clonal preference; etc). Moreover, geographic variability in the performance of different strains of entomopathogenic nematodes further suggests the presence of biotypes; the most virulent strains against banana weevil in Tonga differed from those which effected the greatest weevil mortality in Australia (Gold pers. Com.). Finally, shifting pest status of banana weevil in Uganda from an innocuous herbivore to a serious pest may have resulted from development or introduction of a more successful biotype (Gold pers. Com.). The above examples suggested the possibility of distinct banana weevil biotypes.

Biotyping of banana weevil is critical for understanding variation in pest populations across geographical areas and in different ecological zones. This would provide insight into variability in research results from different locations and will be important in understanding differential response to possible weevil controls.

1.9. Molecular genetics, taxonomy and systematics

Electrophoretic markers are powerful molecular tools that have facilitated the study of diverse areas of biology, both pure and applied, covering questions of phylogeny, evolution, ecology and population dynamics (Loxdale & den Hollander, 1989; Smith & Wayne, 1996; Symondson & Liddell, 1996). Initially, protein markers, especially allozymes, were the main marker type employed: more recently, many of the applications of these markers have been superseded and expanded upon using DNA-based techniques (Avise, 1994). As a consequence, greater resolution has been achieved at the population level allowing the investigation of organisms with little detectable protein variation, e.g. clonal organisms (Carvalho et al., 1991). The minute quantities of DNA required also allows stored samples and specimens with small amounts of organic materials to be studied. All these applications are pertinent to entomology

(Hawksworth, 1994; Hoy, 1994; Crampton *et al.*, 1996; Loxdale *et al.,* 1996; Roderick, 1996; Karp *et al.*, 1998).

The field of molecular biology has expanded greatly in the last ten years and many entomologists now wish to use this technology, since it is a new level of resolution for the study of insect ecological systems and taxonomy. The techniques described include allozyme electrophoresis; restriction fragment length polymorphism (RFLP) analysis; single and multilocus (minisatellite) DNA fingerprinting using various core sequence probes; directed amplification of minisatellite-region DNA (DAMD); sequencing of, for example, mitochondrial DNA (mtDNA), ribosomal cistrons (rDNA) and introns; microsatellites (simple sequence repeats; SSRs); randomly amplified polymorphic DNA (RAPD) markers; amplified fragment length polymorphisms (AFLP); direct amplification of length polymorphisms (DALP); fluorescent in situ hybridization (FISH); reverse transcriptase-PCR (RT-PCR) and PCR-ELISA (enzyme-linked immuno-absorbent assay. In general, the molecular screening described here involved investigating genomic DNA (nuclear, nDNA and mtDNA) or whole chromosomal preparations.

In the case of DNA, it is either purified and digested prior to being probed to detect differences, or techniques such as AFLPs, sequencing and SSRs utilize the polymerase chain reaction (PCR) (Saiki *et al.*, 1998) to amplify regions of interest.

1.9.1. PCR: Principles of Technology

The process of DNA amplification utilizes DNA polymerase and results in the production of large amounts of the target DNA. This process is commonly referred to as the polymerase chain reaction (PCR). The PCR allows the enzymatic amplification of minute quantities of specific DNA sequences. The first experiments with PCR used the Klenow fragment of *Escherichia coli* DNA polymerase (Saiki *et al.*,1985; Mullis and Faloona, 1987). However, activity of the Klenow fragment is diminished by the high temperatures used for denaturing the template DNA. Therefore, more enzyme must be added at the start of each cycle. This was both labour-intensive and expensive. The technique was revolutionized by the discovery of a heat stable polymerase isolated from a thermophilic bacteria, *Thermus aquaticus* and therefore called Taq polymerase (Chien *et al.*, 1976; Saiki *et al.*, 1988). This enzyme is added once at the

beginning of the reaction. The development of programmable thermocycling machines and use of Taq polymerase have made PCR a useful procedure in molecular biology work. The three steps (denaturation, primer annealing and template extension) represent a single PCR cycle. Reaction specificity of PCR is dependent on several factors, which include concentration of primers, template, salts, time allocated for annealing, extension and the total number of cycles. The reaction specificity is enhanced by use of Taq polymerase and hot start of the PCR (Mullis and Faloona, 1987; Erlich *et al.*, 1991). During PCR, each primer becomes incorporated into the PCR product. Mismatch between the primer and the original genomic template DNA can be tolerated (Mullis and Faloona, 1987; Saiki *et al.*, 1988). This allows new sequence information (specific mutation, restriction sites, regulatory elements) and labels to be introduced via primer into amplified DNA fragments (Scharf *et al.*, 1986; Saiki *et al.*, 1988, Erlich *et al.*, 1991). PCR have been utilized in several research applications including the phylogenetic studies of extinct species like the mammoth and the quagga (Paabo *et al.*, 1989). This makes PCR a very useful tool in studying DNA from banana weevil.

1.9.2. Use of RAPD-PCR in genetic and phylogenetic studies

Williams *et al.*, (1991) described a process that they called RAPD (Random Amplified Polymorphic DNA) that uses a ten oligonucleotide primers of random sequence but with a minimum guanine-cytosine contents of 50%. The polymerase chain reaction (PCR) is a highly efficient method of amplifying discrete DNA fragments using a thermostable DNA polymerase and single-stranded DNA primers. An application of the PCR technique that uses DNA primers of arbitrary nucleotide sequence to amplify arbitrary regions of the genome has been described (Welsh *et al.*,1991 and Williams *et al.*, 1991). In order for amplification to occur the primer must anneal on complementary strands of the template DNA and the 3' end of the annealed primers must face each other. The primer annealing sites must be separated at a distance of no greater than 3000 bp as this is the maximum site that can be amplified with routine PCR. In practice amplified fragments are rarely greater than 2000 bp. The requirements that the 3' ends of the annealed primers face one another suggests that annealing sites are exact or similar inverted repeats. The observation that single substitutions, especially in the 3' end of the

primer can change amplified banding patterns (Williams *et al.*, 1991) implies that annealing in RAPD-PCR must be precise.

Inverted and direct repeats occur frequently in the repetitive regions of genomes and these regions are known to undergo rapid changes in size and sequence through unequal crossing over, gene conversion and at the nucleotide level, slippage replication (Levinson and Gutman, 1987). Not surprisingly, arbitrary PCR amplification of these regions reveals, genetic polymorphisms. However, inverted repeats are not confined to repetitive DNA and Williams *et al.*, (1991) reported that unique regions of genomes were amplified as well. Polymorphisms occur as the presence or absence of a specific fragment among individuals. 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 mutations at one or both primer annealing sites on a DNA strand, inversions surrounding a site or insertions that separate the annealing sites at a greater distance than can be amplified. Arbitrarily primed PCR is not quantitative and consequently it is unknown whether individuals whose DNA yields a specific fragment are heterozygous (have one copy) or homozygous (two copies) for an amplifiable allele. Alleles at arbitrarily primed loci therefore segregate as dominant markers (William 1991).

Most alleles of RAPD loci that code for the production of a band are dominant, which makes it impossible to directly estimate allele frequencies. Therefore, assumptions such as Hardy-Weinberg equilibrium must be made to calculate allele frequencies. The dominance of alleles at arbitrarily primed loci presents their greatest drawback for use in insect genetics. Dominance prevents their use in studies of random mating as a component of the breeding structure of natural populations. Testing for conformation to Hardy-Weinberg expectations requires identification of individual alleles at a locus. In common with a number of recent studies (Levi *et al.*, 1993) the present work indicated that while RAPD fragments are useful as genetic markers, a number of other factors can complicate their use and interpretation. The numbers, sizes and intensities of amplified fragments are extremely sensitive to small changes in PCR buffers, co-migration of non homologous fragments, the condition and concentration of template DNA and amplification parameters (Black, 1993).

The relative speed and ease with which these amplified regions, or RAPD (Randomly Amplified Polymorphic DNA) markers can be produced make them suitable for a number of

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applications, including genetic mapping (Paran *et* al., 1991). DNA fingerprinting (Caetano-Anolles *et al.*, 1991) and taxonomic studies (Demeke *et al.*, 1992). RAPD markers have been used to generate molecular markers in a variety of organisms, including humans, fungi and plants (Williams *et al.*, 1991), and more recently, insects (Black *et al.*, 1992).

Among the many molecular methods currently available for taxonomic studies, the random amplified polymorphic DNA (RAPD) polymerase chain reaction technique (Welsh and McClelland, 1990; Williams *et al.*, 1990) appears particularly suitable for population studies of organisms for which only nanogram quantities of DNA are available for characterization. DNA amplification with random primers have been shown to be a sensitive method for revealing polymorphism randomly distributed in any given genome (William *et al.*, 1990; Rafalski *et al.*, 1991). Polymorphism results from either insertions/deletions or substitution of bases at the priming sites (Welsh and McClelland, 1990). The RAPD pattern so obtained have been useful in the derivation of molecular taxonomy and in the study of population genetics of diverse organisms.

The RAPD technique has been used to study a range of insects including, aphids (Black *et al.*, 1992), the honey bee (Hunt and Page, 1992), grasshoppers (Chapco *et al.*, 1992), mosquitoes (Wilkerson *et al.*, 1993) and fruit flies (Haymer and McInnis, 1994). RAPD assays have been used in the characterization of intra and inter specific somatic hybrids of potato (Baird *et al.*, 1992) and genetic mapping of trait(s) of interest such as disease resistance in plants (Rafalski *et al.*, 1991). The analysis of RAPD patterns have been utilized in the identification and differentiation of species and strains of various organisms including *Schistosoma* (Neto *et al.*, 1993; Barral *et al.*, 1993), the sweet-potato whitefly, *Bemisia tobaci* (Perring *et al.*, 1993), *Aedes aegypti* (Ballinger-Crabtree *et al.*, 1992), *T. parva* (Bishop *et al.*, 1993), *T. vivax* (Mohamed *et al.*, 1993), *T. cruzi* (Macedo *et al.*, 1992), *T. brucei* and *T. Congolense* (Waitumbi and Murphy, 1993; Majiwa *et al.*, 1993a), *Leishmania* species (Macedo *et al.*,1992b), Fungus, *Leptosphaeria maculans* which is a pathogen of *Brassica* spp (Godwin and Annis, 1991). In phylogenetic studies, RAPD assays have been used to demonstrate genetic relatedness between species and/or strains of various organisms in relation to their geographical distribution, pathogenecity, host-specificity and other behavioral characters (Chapco *et al.*, 1992; Barral *et al.*, 1993; Ballinger-Crabtree *et al.*, 1992). However, a number of recent studies (reviewed by Black,

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1993) have indicated that, in addition to template concentration, the manner in which DNA is extracted and preserved can also affect RAPD banding patterns (Post *et al.*, 1993).

Statistical analysis of RAPD Patterns can be done using methods similar to those described for RFLP analysis (Nei 1979; Scott *et al.*, 1993). Genetic distance dendrograms or phylogenetic trees can then be constructed from the data using Nei's unbiased genetic distance, similarity index and percentage match analysis (Nei, 1987) with aid of appropriate computer software. The RAPD assay offers a non labour intensive, time saving method of demonstration of genetic relatedness among individuals. Normally, basic procedures such as cloning, probe construction, radiolabelling of DNA and blot hybridization (Southern, 1975; Feinberg and Vogelstein, 1983) are not required. Thus it offers advantages not shared by other methods such as RFLPs and DNA sequencing, currently employed in molecular systematics.

1.9.3. RFLP and the polymerase chain reaction applied to mitochondrial DNA (mtDNA)

Mitochondrial DNA may be purified using a lengthy caesium chloride gradient ultracentrifugation step that allows the separation of the mtDNA from nuclear DNA (White &Densmore, 1992). However more recently, crude preparations of mtDNA have been successful using minipreparations (minipreps) that are performed in much shorter periods (minutes instead of hours) (Latorre et al., 1986). Mitochondrial DNA separated in this way has been digested and visualized by radio end-labelling (Smith & Brown, 1990). The polymerase chain reaction has also been used to amplify specific parts of the mtDNA from such preparations. PCR has increased the scope of material that can be used for DNA studies. Small amounts of DNA can be used from mixed templates of nuclear DNA (nDNA) and mtDNA as a source of investigation, as specific primers will only amplify from select, known regions of DNA in stringent PCR reactions. DNA can be extracted from alcohol-stored material and even, in some instances, dried museum specimens (Wilson *et al.*, 1985; Paabo, 1993) and from ancient insects held in amber (DeSalle, 1994; Grimaldi, 1996).

Universal primers have been developed from conserved sequences developed from conserved regions flanking those of interest to phylogenetic / evolutionary and population studies (Simon *et al.*, 1994). Such primers allow the amplification of the same region from a diverse range of taxa facilitating parallel research studies in new organisms. Briefly, eukaryotic organisms have a circular mitochondrial DNA molecule (Roehrdanz, 1993, 1995). The molecule

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is made up of 37 genes coding for 22 tRNAs, 2 rRNAs and 13 mRNAs: most of the mitochondrial DNA is involved in coding, with general lack of introns, large families of repetitive DNA, pseudogenes and large spacer sequences. The mitochonrial DNA consist of the following major coding regions in sequence: ND2, CO1, CO2, ATP, C3, N3, ND5, ND4, ND6, Cyt b, ND1, 16S, 12S and A+T regions. Mitochondrial DNA is a valuable marker to indicate maternal gene flow as it is predominantly transmitted through maternal lines (Avise, 1991, 1994). Animal mtDNA is therefore the best known piece of eukaryotic DNA because it is easier to purify than any specific segment of nuclear DNA. This ease of purification is the result of an unusual buoyant density, a high copy number, and occurrence in an organelle other than the nucleus. MtDNA is also easy to characterize because it is small and lacks many of the complicating features of nuclear DNA (such as introns and repetitive sequences). The types of evolutionary change that animal mtDNA undergoes are relatively simple, being mainly base substitutions and length mutations, the latter accumulating predominantly in the small non-coding regions. When suitable large products of PCR amplification are purified using commercially available kits and digested with endonucleases to reveal polymorphism in an amplified product it is termed RFLP-PCR. DNA extracted from small amounts of tissue can be used for molecular analysis using polymerase chain reaction (PCR) and restriction fragment length polymorphisms (RFLPs) (Crozier, 1993). PCR-RFLP has been successfully used for molecular diagnostics of gypsy moth (Pfeifer, 1995), blow flies (Sperling *et al.,* 1994), screworm (Taylor *et al.*, 1996) and Muscidifurax spp (Taylor and Szalanski, 1999, Taylor *et al.*, 1997 and Antolin *et al.*, 1996).

1.9.4. Data analysis

RAPD-PCR polymorphism is analyzed as alleles by making four assumptions (Black, 1993). First RAPD products segregate as dominant alleles in a Mendelian fashion. Secondly, genotype frequencies at RAPD loci are in Hardy – Weinberg proportions. Thirdly, alleles in a homozygous recessive individual are identical in state (iis) (i.e. that they arose from identical mutations) among and within individuals. Fourthly, dominant, amplified alleles are similarly (iis). RAPDs are scored as present (1) or absent (0). The frequency of recessive alleles "a" is estimated as square root of the frequency of homozygous recessive individuals i.e. if "q" is the frequency of the "a" allele then "1-q=p" is the frequency of the dominant allele "A".

FORTRAN programme DISPAN (Genetic Distance and Phylogenetic Analysis) (Tatsuya, 1993) uses the frequency data to output a distance matrix according to Nei, 1972. This matrix is used in cluster analysis with an option of UPGMA (unweighted pair group method, arithmetic average) to construct a dendrogram that depicts the hierarchical structure of RAPD affinity among the different weevil populations.

1.9.4.1. Measuring Genetic Diversity

The following three measures are used to quantify the amount of genetic variability within a population.

1. The percentage of polymorphic loci is the proportion of loci polymorphic within its population (Lin *et al.*, 1997).

2. Genetic diversity (h) at each RAPD locus in each population is determined using the method of Nei (1973). Genetic diversity within each population is calculated as the mean genetic diversity over all loci from all populations.

3. The genetic diversity is estimated by the Shannon information index of phenotypic diversity: $H_o = -\Sigma P_i \log_2 P_i$, where P_i is the frequency of phenotype i (King and Schaal, 1989). H_o can be calculated and compared for different populations. Let $H_{pop} = (\Sigma H_o)/n$ be the average diversity over n different populations and let $H_{sp} = -\Sigma P \ln P$ be the diversity calculated from the phenotypic frequencies, P, in all populations considered together. Then, the proportion of diversity present within populations, H_{pop}/H_s, can be compared with that between populations $(H_{sp} - H_{pop})/H_{sp}$ (Gustafsson & Gustafsson, 1994).

1.9.4.2. Genetic differentiation

Three methods are used to quantify the degree of differentiation among populations.

1. The genetic divergence among populations is estimated by calculating Nei's Gst value (Nei, 1987).

2. Gene flow (Nm) represents the actual number of individuals exchanged among populations in each generation. Gene flow is quantified using the indirect estimates of Crow and Aoki (1984) after Ellstrand and Elam (1993). In this model, the number of migrant individuals exchanged among demes of each generation is Nm = (1/Gst –

$1)/4[n/(n-1)]^2$, in which n = total number of demes, using the average value of Gst over all loci.

3. Genetic divergence among populations is estimated using the method of Nei (1978). Genetic distance values (D) for all pairs of populations are used to construct a dendrogram by the unweighted pair group method with arithmetic averaging (UPGMA) using DISPAN.

Chi-square analysis tested the independence of pairwise associate between bands amplified by the same primer and across primer. The G^2 likelihood ratio chi-square test (Test of goodness of fit) was estimated using $G^2=2\Sigma((\log (pyi) - \log (Pyi))$ where pyi is the hypothesized probability and Pyi is the estimated probability for the events yi that actually occurred.

1.9.5. Importance of study of Genetic diversity in banana weevil.

The banana weevil, *Cosmopolites sordidus*, is by far the most important biotic constraint to banana production in many banana growing regions. The importance of bananas as staple and commercial crops in sub-Saharan Africa, Latin America, Asia, the Pacific and Australia underscores the need for sustainable ways of dealing with the problems posed by this pest

The importance of studying intra-species variability goes far beyond the realm of basic genetics. In the case of banana weevil, insight into the existence of biotypes has several levels of application. The first is extrapolation of research results and an understanding of the inconsistencies found between similar studies. How relevant is a study conducted in Latin America or West Africa to the East African context? Are there differences in weevil biology between lowland and mid-elevation banana systems? Are weevils found attacking dessert bananas, similar to those attacking highland cultivars? Awareness of how much genetic variability exists among local populations within and between geographic regions will help both in the interpretation of studies on weevil biology while also contributing to a better understanding of population dynamics, habitat characteristics, dispersal and distribution patterns, resource utilization, and other ecological processes. For example, if high levels of variability exist in East Africa, then critical studies may need to e undertaken in the region, irrespective of the information available from studies conducted elsewhere. Secondly, the existence of biotypes may suggest the need for multilocation testing with respect to resistant germplasm, to different strains of fungal pathogens and entomopathogenic nematodes or to predator prey studies with

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weevil natural enemies. Differences in pest behavior may influence efficacy of biological control agents; for example, oviposition sites may determine vulnerability of the egg stage to egg parasites. Also, the responses to different semiochemicals may vary between weevil populations and this will in turn influence the efficiencies of any trapping or monitoring devices that may be developed. It is for these reasons that this project proposes to examine the genetic variability among banana weevil populations from the major banana growing regions of the world. At the end of the project, it is hoped that a clear picture regarding the genetic variability of this important pest will have emerged.

1.9.6. Objectives

1.9.6.1. Overall Objective

The overall objective of this project is to study the genetic diversity in banana weevil populations from different banana growing regions of the world and to evaluate the implications of this diversity on management strategies and quarantine.

1.9.6.2 Specific objectives:

(I)   To explore how much diversity exists within & among the banana weevil populations from different regions of the world and Uganda using RAPD.

(II)   To develop suitable molecular markers of randomly amplified polymorphic DNA (RAPD) for studying banana weevils from different banana growing regions of the world.

(III)   To assess population genetic structure of banana weevils from different regions of the world and Uganda based on information obtained under activity one.

(IV)   To analyse of intra-specific differentiation of banana weevils in the tropics in relation to their histories and zoogeography.

(V)   To analyse intra-specific differentiation to derive information on taxonomy and phylogenetic relationships.

(VI)   To compare PCR-RFLPs within the COI mitochondrial region of *C. sordidus* from different geographic regions to estimate inter-species group divergence and to assess restriction profiles as potential genetic markers.

2.0. Materials and Methods

2.1. Weevil collection from different geographical areas

Intact adult weevils (males and females) were collected from different banana growing regions of the world (fig.3) and different banana growing regions of Uganda (fig.4) and shipped to the International Centre of Insect Physiology and Ecology (Molecular Biology and Biochemistry Department) in 70% ethanol.

Figure 3:

Banana weevil collection sites from different regions of the world.

Everglades, South Florida (USA)

Guarabo (Puerto Rico)

Corte (Honduras)

Guadalupe (Costa Rica)

Manizales (Colombia)

Kade (Ghana)

Njombe (Cameroon)

Ntungamo (West Uganda)

Mbita, Lake Victoria (Kenya)

Embu, Central (Kenya)

Bukoba District (Tanzania)

Pemba (Zanzibar)

Sabie River Valley, Mpumalanga (South Africa)

Solok, West Sumatra (Indonesia)

TFRS, New South Wales (Australia)

● Sampling Site
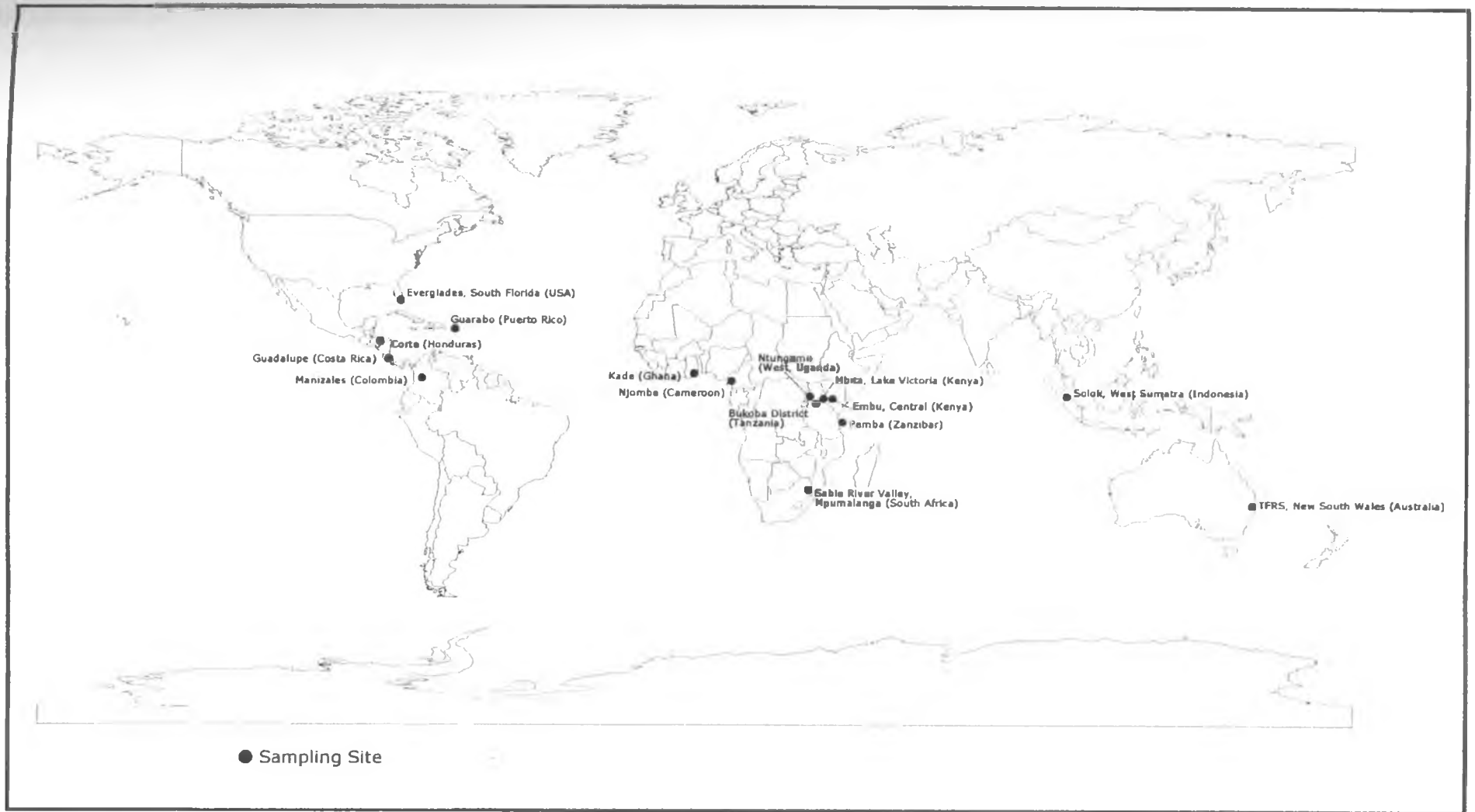
Figure 4:

Banana weevil collection sites in Uganda. Some sites are points, others are districts. This is exactly the information given by samplers.
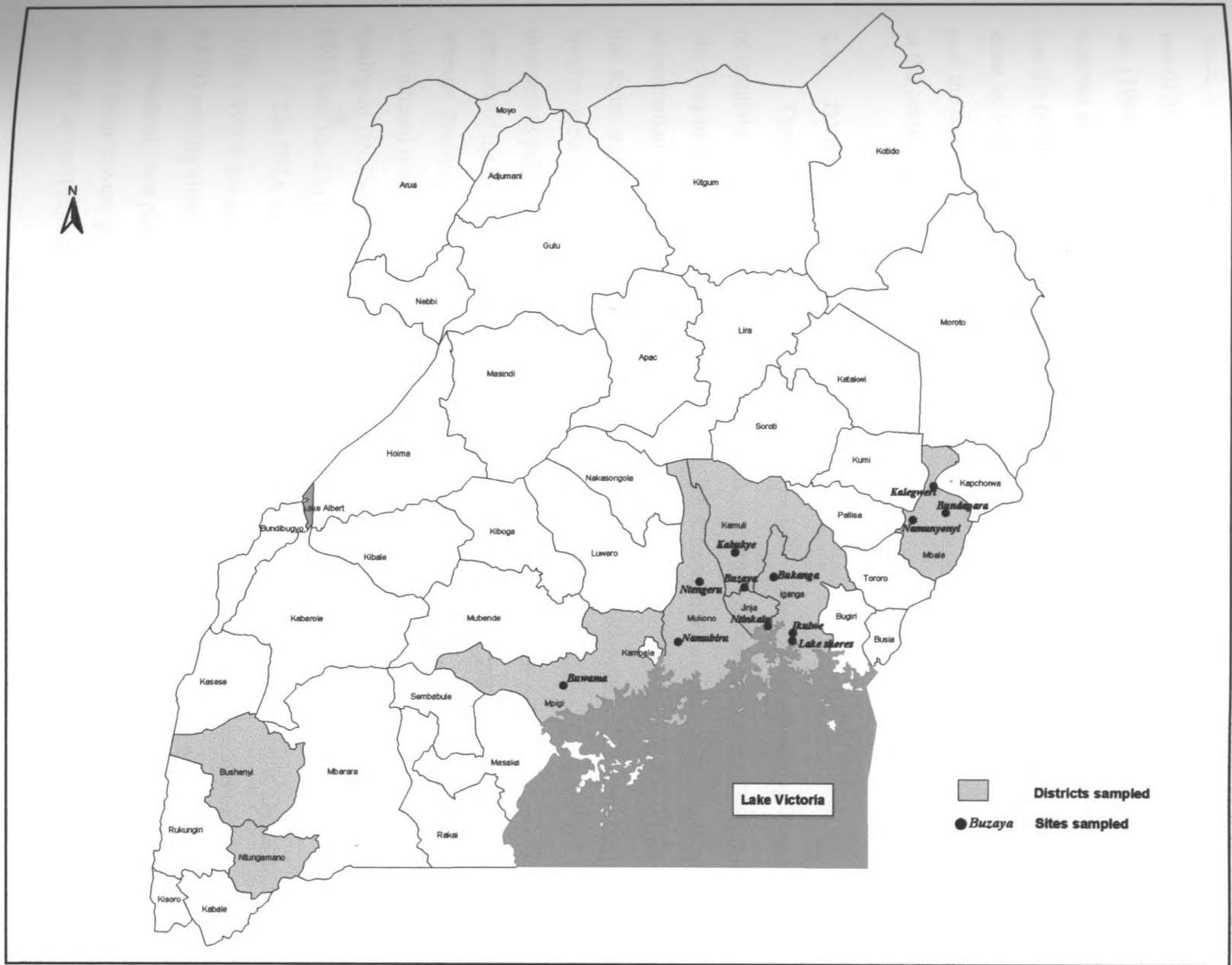
2.2. Sexing of weevils

Only male weevils were used for the experiment. This avoided contamination of a
DNA with DNA from eggs and reduced the possibility of contamination from ingested pr
since, unlike females, males do not require a proteinaceous meal prior to reproduction. W
use of males alone could have limited the degree of genetic variation observed, work by F
al., (1980) indicated that more than 95% of the genetically variant loci detected in *Lucilia*
cuprina dorsalis Robineau-Desvoidy are autosomal. Hence, any study of one or other se
has the potential to cover the significantly major proportion of all genetic variation. Sexi
done by looking at the proboscics (Zimmerman, 1968). If the white dots in the proboscic
past the center, measuring from the head towards the tip, then that was a female. If the d
at the center or before then, it was a male.

2.3. DNA extraction

There is a wide variety of problems to consider during DNA extraction. Problem
of template quality and concentration as well as extraction of contaminating DNA from
other organisms. Extraction of contaminating DNA from other organisms (e.g. bacterial
or protozoan endosymbionts) with DNA of the target taxon is inevitable and it is likely
that some amplified bands will arise from the contaminating template. If contaminating
species occur sporadically throughout the target taxon or if the contaminants are
themselves polymorphic, the amplified bands can be mistaken for polymorphisms in th
genome of the target taxon. Hence DNA was extracted from the head and legs of banan
weevils. Problems of template quality and concentration were overcome by use of the
cold ethanol preservation technique and the extraction protocol described. The yield an
quality of DNA extracted from inidividual weevils was therefore assessed and only int
DNA was used in the study.

The DNA isolation was performed on individual samples according to Doyle ar
(1987) with some modification. Sample size of weevils from each site are indicated in
RAPD scoring sheet in the appendix. Briefly, 0.125 grams of the banana weevil (head
appendages) were placed in a 1.5 ml eppendorf tube. Using plastic disposable grinder
weevil sample were ground up in the eppendorf tube at room temperature, without buf
lo 20 sec or until liquid exuded from the tissue. 250 ul of CTAB extraction buffer (50

pH 8.0, 10 mM EDTA, 0.7 N NaCl, 1.0% Cetyl-trimethyl ammonium bromide (CTAB) and 0.1% β-mercaptoethanol) pre warmed to 60°C was added and inverted several times to disperse the lamp. This was then incubated at 60°C for 15 min with occasional mixing by inversion. 250 ul of chloroform was added and mixed by inversion until an emulsion was formed followed by centrifugation at 13,000 rpm in a table-top centrifuge for ten minutes at room temperature. The aqueous phase was placed into a fresh eppendorf tube followed by chloroform addition and centrifugation as described above. The clear phase was transferred to a fresh tube and 0.6 volumes of isopropanol added and mixed by inversion to precipitate the DNA. This was left at room temperature for 10 min and then centrifuged at 13,000 rpm in a tabletop centrifuge for 10 min at room temperature. The liquid was removed carefully without disturbing the pellet. 250 ul of ethanol was added and mixed vigorously by inversion several times and centrifuged at 13,000 rpm in a tabletop centrifuge for 5 min at room temperature. The liquid was carefully removed without disturbing the pellet and vacuum dried for 15 min and the DNA resuspended in 50 ul of TE(10 mM Tris-HCl pH 8.0, 1 mM EDTA). The yield and quality of DNA extracted was assessed on a 0.8% Tris-borate EDTA (0.5 x: 45 mM Tris-borate, 1 mM EDTA pH 8.0) agarose gel prior to RAPD characterization and the DNA was assayed by measuring UV absorbance in 1:1000 dilution in TE at 260 nm .

2.4. RAPD-PCR amplification

2.4.1. Primers

Forty primers (Operon 10-mer Kits A and O) (Operon technologies, Alameda, CA, USA) were screened, of these 5 produced discrete band patterns suitable for subsequent analysis of variability in weevil populations.

2.4.2. PCR amplification

All reaction tubes, pipette, tips and water (distilled-de-ionised and autoclaved) were irradiated with UV light to destroy any possible contaminating surface DNA (Gawell and Bartlett, 1993). RAPD PCR reactions were set up in a final volume of 25 µl containing 10 ng of the genomic DNA of the banana weevil, *Cosmopolites sordidus*, 5 µl of single decamer primer (5

picomoles/ μl), 2.5 μl of 10 x PCR buffer (50 mM KCl, 10 mM Tris-HCl pH 8.4, 1 mg gelatin/ml), 2 μl of 25 mM $MgCl_2$, 1 μl of 5 mM dNTP, 0.1 ul Taq DNA polymerase (2 units) (Strategene, USA) in a 0.5 ml sterile microfuge tube. The final volume was made up with sterile water. The content was spun down briefly and one drop of mineral oil added to each reaction tube to prevent evaporation of the content during PCR reaction. Positive and negative control tubes were included in the reaction and contained the reaction mix with DNA that successfully amplified before and without any DNA templates respectively. All the reactions were assembled assceptically in a laminar flow hood. The reaction was carried out in a Hybaid Omn-E thermal cycler (Midwest Scientific St. Louis, USA), programmed to denature at 94°C for 1 minute, anneal at 36°C for 1 minute and extend at 72°C for 2 minutes. Amplification was continued for 45 cycles with final extension at 72°C for 10 minutes. The reaction was terminated by cooling to 4°C until when collected (Innis *et al.*, 1990). The RAPD products were separated by electrophoresis at 2.7 V/cm in 1.2% agarose gels using 0.5x Tris Borate-electrophoresis buffer (TBE) pH 8.0. DNA fragments were visualized by staining with ethidium bromide (5 ug/ml) and photographed on a 310 nm UV transilluminator (Sambrook *et al.*, 1989).

From the above results, genetic distance matrix was developed and used to construct UPGMA cluster.

2.5. Extraction and purification of polymorphic bands

Samples clustered into six clusters from the UPGMA constructed. All the DNA for every cluster was pooled up. Amplification reactions were performed in volumes of 100 μl for each cluster containing 1x PCR buffer (10 mM Tris-HCl, pH 8.4, 50 mM KCl, 1mg gelatin/ml), 2 mM $MgCl_2$, 200 mM each of dATP, dCTP, dGTP and dTTP (Pharmacia), 25 picomoles primer, 10 ng of genomic DNA and 2 units of Taq DNA polymerase (Strategene). Amplification was performed in a Hybaid Omn-E thermal cycler (Midwest Scientific St. Louis, USA). Thermal Cycler programmed for 45 cycles of 1 minute at 94°C, 1 minute at 36°C, 2 minutes at 72°C and a final extension at 72°C for 10 minutes. Amplifications were analysed by electrophoresis in 1.2% agarose gels run at 80 V (constant) for 1.5 hours in 0.5 x TBE buffer and detected by staining with ethidium bromide.

The polymorphic bands were extracted according to Qiaex DNA Gel Extraction (Experienced User Protocol) with some modification. Briefly 126 ml or 420 ml of ethanol (96-

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100%) was added to the QX3 concentrate to obtain 180 or 600 ml of buffer QX3, respectively. The DNA fragment was excised and to it was added 300 µl of QX1 per 100 mg of the gel. Qiaex was suspended and 10 µl of it was added and mixed, followed by incubation for 10 min at 50°C. This was centrifuged at 4,000 g for 1 min. The pellet was washed twice in 500 µl of QX2 and then twice in 500 µl of QX3. The pellet was air dried and the DNA eluted in 20 µl TE.

2.6. Cloning of polymorphic bands:

2.6.1. Ligation

The ligation reaction was done using the PCR product and the PGEM-T Easy vector (Promega Corporation, Madison, U.S.A) at concentrations of 25 ng/ml and 50 ng/ml respectively. The ligation reaction was set up in a total volume of 10 µl as follows: 3 µl PCR product, 5 µl of 2 x ligation buffer (60 mM Tris-HCl (pH 7.8), 20 mM $MgCl_2$, 20 mM DTT, 2 mM ATP, 10% polyethylene glycol), 1 µl T4 DNA ligase (3 Weiss units/µl) and 1µl pGEM-T vector. The ligation mixture was incubated at room temperature for 1 hour. 4 µl of the ligation reaction was used to transform competent bacterial cells.

2.6.2. Preparation of competent bacterial cells.

Tubes of frozen JM 109 High Efficiency competent cells were removed from -70°C storage and placed in an ice bath until just thawed (about 5 minutes). The cells were mixed by gently flicking the tube. The cells were streaked and grown in an overnight liquid culture in 2 x YT medium (YT medium: 16 g/l Bacto-tryptone, 5 g/l Nacl, 10 g/l yeast extract). Two ml of the overnight culture was inoculated into 50 ml of 2 x YT in 250 ml conical flask. The culture was incubated at 37°C in thermal shaker at 225 rpm until the cell density reached between 6-9 x 10^7 viable cells/ml (OD_{600} = 0.4-0.6). The culture was then transferred to a sterile polypropylene tube and chilled on ice for at least 10 minutes. The cells were pelleted by centrifugation at 2,500 rpm at 4°C for 15 minutes. The medium was decanted and the cells were resuspended in 1/10 th of the original volume of cold sterile 0.1 M $MgCl_2$ (5 ml). This was left in ice for 20 min, repelleted by centrifugation and resuspended in 1/20th of the original volume of 0.1M $CaCl_2$ on ice (2.5 ml), mixed and kept in ice for a minimum of 30 minutes. The cells were used immediately.

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2.6.3. Transformation of bacteria

The procedure described by Sambrook *et al.*, (1989) for transformation of bacteria was used. The competent cells in ice were gently mixed by tapping the tube. The cells (200 μl) were aliquoted into prechilled Falcon 2059 polypropylane tubes. 4 μl (0.1-50 ng) of the ligation reaction mixture was added to each tube. The resultant mix was swirled gently and then kept in ice for 5-10 minutes. The tubes were then placed in a water bath at 42°C for 1.5 minutes, to heat-shock the cells and then immediately chilled on ice for 2 minutes. The cells were plated on NZYCM (10 g/l NZ amine, 5 g/l NaCl, 1 g/l Casamino acid, 1.71 g/l $MgSO_4 7H_2O$, 12.06 g/l Agar) plates containing 20 μl of 2% IPTG, 35 μl of 10 mM X-gal and 50 μg ampicillin/ml. The plates were incubated at 37°C overnight in an inverted position. Putative recombitant bacterial colonies, containing insert PCR products were identified by colour selection. White colonies were scored and picked with sterile toothpick under lamina sterile hood. PCR screening of the white colonies was done using PGEM-T primers in a total volume of 50 μl in a Hybaid Omni-E thermal cycler (Midwest Scientific St. Louis, USA) as follows: 1 μl of 10 mM dNTPs, 0.5 μl Taq (5 U/μl), 1 μl reverse primer, 1 μl forward primer, 5 μl of 25 mM $MgCl_2$, 5 μl 10 x PCR buffer, 36.5 μl H_2O. PCR condition was as follows: Denaturation 94°C/min, annealing 55°C for 30 seconds, extension 72°C for 2 min and a final extension at 72°C for 10 min, number of cycles 30. Two samples were picked from each plate.

2.6.4. Maintenance of recombinant clones

The clones were picked and grown overnight in a thermal shaker at 37°C, 225 rpm in 5 ml of 2 x YT medium supplemented with 50 μg ampicillin/ml. Isolation and purification of the recombinant plasmids was carried out by small scale DNA preparation.

2.6.5. Small scale plasmid DNA preparation (Miniprep)

Isolation and purification of the plasmid DNA was carried out using Wizard SV minipreps Kit (Promega Biotech, Inc., USA). Briefly, cells from the 5 ml antibiotic culture were harvested by centrifugation at 14,000 rpm for 30 sec in a microcentrifuge. The cell pellets were suspended on 200 μl cell suspension solution (500 mM Tris-HCl pH 8.0, 10 mM EDTA, 100

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μg/ml RNAse) followed by vigorous vortexing. The suspended cells were transferred to an eppendorf tube. 200 μl cell lysis solution (0.2 M NaOH, 1% SDS) was added and mixed by inverting the tube several times until the cell suspension became clear. 200 μl neutralization buffer (2.55 M Potassium acetate, pH 4.8) was added immediately and mixed by several tube inversions. This was spun at 14,000 rpm for 5 min in a minicentrifuge and the supernatant decanted to a new eppendorf tube. 1 ml of DNA purification resin was added and mixed with the solution by several tube inversions. Magic minicolumn was then prepared by removing the plunger from a 3 ml disposable syringe and the syringe barrel attached to the minicolumn extension. Resin/DNA mixture was loaded into the syringe barrel and the slurry pushed gently into the minicolumn. The syringe was detached from the minicolumn and the plunger removed from the syringe. 2 ml of the column wash solution was pippeted into the syringe and gently pushed through minicolumn. The minicolumn was transferred into a new 1.5 ml microfuge tube and centrifuged at 14,000 rpm for 20 sec in microcentrifuge to dry the resin. The minicolumn was transferred to a new tube and 100 μl sterile nuclease-free water applied. The DNA was eluted after 1 min by spinning the minicolumn content at 14,000 rpm for 20 seconds. The recombinant plasmids were checked by loading 5 μl on a 1% agarose gel. Presence of inserts were confirmed by digestion with ECORI. Digestion reactions were carried out in small tubes as follows; 2 μl (10 weiss units) ECORI restriction enzymes (Boehringer Mannheim, Germany), 2 μl of 10 x reaction buffer (50 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 100 mM NaCl, 1mM Dithioerythritol (DTE)), 1.0% Triton X100, 5 μl purified plasmids and 11 μl of double distilled water. The tubes were incubated for 1 hour at 37°C in a water bath. After digestion, 15 μl of the mix was resolved in 1.2% agarose gel. The true recombinants plasmid ligated with insert were scored and stored at 4°C (Sambrook *et al.*, 1989).

2.6.6. Sequencing and Design of primers

Samples were sent to the International Livestock Research Institute (ILRI) for sequencing and synthesis of primers.

2.6.7. Polymerase chain reaction (PCR) using synthesized primers

PCR amplifications of DNA of individual clusters were carried out in a volume of 25 μl as follows: 2.5 μl of 10 x PCR buffer (500 mM KCl, 100 mM Tris-HCl pH 8.4, 1 mg gelatin/ml), 0.25 μl of 25 mM MgCl$_2$, 2.0 μl of 5 mM dNTPs, 0.1 μl Taq (5 U/μl) Strategene), 2 μl of genomic DNA (10 ng), 1 μl (0.3 μm) reverse and forward primers and 16.15 μl water. The contents were spun down briefly and 1 drop of mineral oil was added to the reaction. Amplification was carried out in a Hybaid Omni-E thermal cycler (Midwest Scientific St. Louis, USA) programmed to denature at 94°C for 1 minute, followed by 34 cycles of 94°C for 15 seconds, 53°C for 10 seconds, 72°C for 20 seconds and finally 1 cycle at 72°C for 4 minutes.

2.7. Labeling and detection with the DIG system

2.7.1. Standard random primed DNA labeling of purified polymorphic DNA fragment reaction.

DNA labeling was done by random priming according to the DIG User's Guide for Filter Hybridization (Mannheim Boehringer, Germany). The reagents were added to a sterile microfuge tube (on ice) in the following order: 10 μl DNA template (10 ng-3 μg), 2 μl Hexanucleotide mixture (10 x), 2 μl dNTP labeling mixture (10 x), 5 μl H$_2$O and 1 μl Klenow enzyme, labeling grade. The DNA template was heat denatured in a boiling water bath for 10 minutes and immediately chilled in dry ice for 30 seconds before use. The reaction mix was incubated at 37°C for 20 hours. 2 μl EDTA (200 mM EDTA, pH 8.0) was added to terminate the reaction. DNA was precipitated with 0.1 volumes of 4 M LiCl and 2.5 - 3 volumes chilled ethanol, mixed and incubated at -70°C for 30 minutes. The content was centrifuged at 13,000 g in a microcentrifuge at 4°C. Ethanol was decanted and the pellet washed with 100 μl chilled 70% ethanol. This was centrifuged at 13,000 g for 5 minutes at 4°C. The pellet was dried and resuspended in 50 μl TE (10 mM Tris-HCl, 1 mM EDTA; pH 8.0) buffer and the labelled probe stored at -20°C until use.

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2.7.2. Southern blotting of DNA to nylon membranes

2.7.2.1. Agarose gel electrophoresis

DNA samples for each cluster were pooled and PCR reactions were carried out in a total volume of 25 µl in a Hybaid Omni-E thermal cycler (Midwest Scientific St. Louis, USA) as follows: 2.5 µl of 10 x PCR buffer, 0.5 µl of 25 mM $MgCl_2$, 2 µl of 2.5 mM dNTPs, 0.1 µl Taq polymerase (5 U/µl) (Strategene), 2 µl of DNA (10 ng), 5 µl primer (5 pic/µl) and 12.9 µl of water. Amplification was carried out as follows: A total of 45 cycles, 95°C for 1 minute, 36°C for 1 minute, 72°C for 2 minutes and a final extension at 72°C for 10 minutes. PCR product was run in a 1.2% agarose gel 80 V (constant) for 1.5 hours using, 0.5 x Tris Borate- electrophoresis buffer (TBE) pH 8.0. DNA fragments were visualized by staining with ethidium bromide (5 µg/ml).

2.7.2.2. Denaturation, neutralization and blotting

The agarose gel was submerged in denaturation solution (0.5 N NaOH, 1.5 M NaCl) twice for 15 minutes at room temperature with shaking. The gel was rinsed with water. The gel was submerged in neutralization solution (0.5 M Tris-HCl, pH 7.5; 3 M NaCl) twice for 15 minutes at room temperature. Membrane filters for Southern transfer were prepared according to (Sambrook, et al., 1989). The actual pH of the gel after neutralization was checked (it should always be below pH 9.0 otherwise membranes turn yellow and break during hybridization) (DIG User's Guide for Filter Hybridization, Mannheim Boehringer, Germany). DNA was blotted from the gel by capillary transfer to the membrane using 20 x SSC buffer (3 M NaCl, 300 mM Sodium citrate, pH 7.0) as follows: The gel was placed on top of three layers of Whatman 3 MM filter paper (soaked with 20 x SSC and placed on top of an inverted gel casting tray in a plastic box such that the filter paper did not touch the base of the box , forming a wick), and was laid with a nylon membrane and three layers of filter papers, all of which were soaked in 20 x SSC. A thick wad of dry tissue paper was placed on the gel/membrane/filter paper sandwich, followed by another glass plate and weight. Blotting was carried out by capillary transfer to the membrane using 20 x SSC buffer. For efficient transformation, blotting was carried out overnight. The DNA was fixed on to the membrane by baking in an oven for 2 hours at 80°C. The membrane was used for hybridization immediately or stored at 4°C in a sealed plastic bag.

2.7.2.3.   Prehybridization and hybridization

The blot was placed in a hybridization bag containing 20 ml standard prehybridiaztion solution (5 x SSC, 1.0 % W/V blocking reagent, 0.1 % N- lauroylsarcosine, 0.02% SDS) per 100 cm² of membrane surface area. The bag was sealed and prehybridized at 68°C overnight. DNA probe was heated in a boiling water bath for 10 minutes and chilled immediately on ice. The probe was diluted in standard hyridization solution to a probe concentration of 5-25 ng/ml. The amount of standard hybridization solution for 10 x 10 cm blot was at least 3.5 ml. The prehybridization solution was discarded and the hybridization solution containing the DIG-labeled probe added and left to hybridize overnight. At the end of the hybridization, the hybridization solution was poured into a capped tube and frozen for future use. The membrane was washed twice 5minutes per wash in 2 x wash solution (2 x SSC containing 0.1 % SDS). The membrane was then washed twice, in 0.1 x wash solution (0.1 x SSC containing 0.1% SDS) for 15 minutes per wash at 68°C.

2.7.3.   Colorimetric detection with NBT and X-phosphate

All the reactions were done at room temperature with shaking. After hybridization and post hybridization washes the membrane was equilibrated in filtered buffer 1 (100 mM maleic acid, 150 mM NaCl, pH 7.5) for 1 minute. The membrane was blocked by gently agitating in buffer 2 (1% V/W blocking reagent dissolved in buffer 1) for 30 minutes in a plastic bag. The anti DIG-alkaline phosphatase was diluted in a ratio 1:5,000 in buffer 2 to a working concentration of 150 mU/ml (6 µl Anti DIG-alkaline phosphatase was added to 30ml buffer 2 and mixed) (DIG User's Guide for Filter Hybridization, Mannheim Boehringer, Germany). This working antibody solution is stable for about 24 hours at 4°C. The membrane was incubated in the antibody solution for 30 minutes. The membrane was washed twice in a sufficient amount of Buffer 1 for 15 minutes per wash. The membrane was equilibrated in 20 ml buffer 3 (100 mM Tris-HCl, pH 9.5, 100 mM NaCl, 50 mM MgCl₂) for 2 minutes. The colour substrate was prepared by adding 45 µl NBT solution and 35 µl X- Phosphate in 10 ml of buffer 3. This solution was added directly to the membrane in a sealed bag in the dark. The formation of the color precipitate started within a few minutes and the reaction was complete within 12 hours. Once the desired bands had been detected the membrane was washed with 50 ml of Buffer 1 for 5 minutes to stop the reaction.

2.8. PCR-RFLP analysis of the CO1 region of the mitochondria

2.8.1. Samples

Samples used were obtained from Pemba, Australia (Murlimbugah), Thailand, South Africa, Mbita, Honduras, Embu, Costa Rica (Mag Diamanted), Indonesia, Zanzibar, Costa Rica (Finca Guadaloupe), Australia (Coffs), Ghana, Tanzania, Cameroon, Uganda (Masaka) and Uganda (Kawanda), Columbia and Puerto Rico.

2.8.2. DNA extraction

Pools of 5-25 weevils were used for each DNA extraction. Total DNA was isolated fro appendages and head using the CTAB method (Doyle and Doyle, 1989).

2.8.3. PCR amplification

The CO1 mitochondrial region was amplified using the two pairs of primers S1460: TACAATTTATCGCCTAAACTTCAGCC and A2442: GCTAATCATCTAAAAACTTTAATTCCWGTWG and S2442: CCAACAGGAATTAAAATTTTTAGATGATTAGC and A2771: GGATARTCAGARTAACGTCGWGGTATWC (Primers were supplied by Brian D. Farrell, Harvard University). PCR reactions were carried out in a total volume of 25 μl in a Hybaid Omni-E thermal cycler (Midwest Scientific St. Louis, USA) as follows: 2.5 μl of 10 x PCR Buffer, 2 μl of 5 mM dNTPs, 0.1 μl Taq (5 U/μl) (Strategene), 1.0 μl BSA (1 mg/ml) (Sigma μl genomic DNA (10 ng), 15.4 μl water and 1 μl (0.3 μM) reverse and forward primers. The PCR profile was 95°C for 1 minute followed by 39 cycles of 95°C for 30 seconds, 45°C for 1 minute, 72°C for 1.5 minutes and finally 1 cycle at 72°c for 10 minutes.

2.8.4. Restriction Endonuclease digests.

Amplicons were digested with 51 restriction enzymes – Alu I, Ava I, Ava II, Avi II, Bgl I, Bgl II, BstE II, BmH I, BssHI, Cla I, Cfo I, Dra II, Dra III, Dde I, Dpn I, Dra I, EcoR I, Eco47 III, EcoR V, Fok I, Hae II, Hae III, Hinf I, Hind II, Hind III, Hpa I, Hpa II, Kpn II, Ksp I, Msp I, Mlu I, Nae I, Nde II, Nci I, Not I, Nru I, Nsi I, Pvu I, Pvu II, Rsa I, Sac I, Sal I, Sau I, Sca I, Sfi I, Sma I, Spha I, Taq I, Xba I, Xho I, Xho II. Twelve enzymes Ava II, BstE II, Dra II, Hae III, Dde I, Hinf I, Hind II, Hpa II, Nde II, Rsa I, Sau I and Xba I cut the amplicon generated from S1460 and A2442 (1000bp) and only one enzyme Alu I cut the amplicon from S2442 and A2771 (400bp). The PCR product (1000 bp) was therefore digested with BstE II, Hae III, Dde I, Hpa II, Nde II, Rsa I, Xba I and Hinf I. The PCR product (400 bp) was digested with Alu I. All restriction digests were carried out according to the manufacturer's instructions (Mannheim, Boehringer, Germany). The fragments were separated on 1.5% agarose gels (1x TBE buffer) and visualized with ethidium bromide and ultraviolet light.

3.0.   RESULTS

3.1.   Genetic diversity within and among the banana weevil populations from different regions of the world

3.1.1. DNA Extraction

To avoid contaminating DNA sample with DNA from ingested food or gut parasites, only the head and legs of male *C. sordidus* were used as sources of DNA. After verifying that these tissues gave identical amplification patterns, tissues were combined when extracting DNA from individual weevils. DNA was extracted, as total nucleic acid by the cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle 1987). The quality of DNA obtained, the suitability of CTAB extraction for material rich in polysaccharides and the small number of extraction steps required, made this method ideal for preparation of the small quantities of DNA present in chitinous insect tissues. Approximately 2000 ng of DNA was obtained from the head and legs of individual weevils.

The yield and quality of DNA extracted from field individual weevils was assessed using a 0.8% tris-borate-EDTA (TBE) agarose gel prior to RAPD characterization. Samples (5µl of total nucleic acid) were run against 2µl of DNA molecular weight marker XIV (Boehringer Mannheim). The discreteness of the high molecular weight band (7943bp) and the degree of streaking in each sample lane provided a measure of DNA quality (fig.5).

The suitability of absolute ethanol for preservation was evaluated by storing *C. sordidus* for 0, 2, 4, 6 and 8 weeks in absolute ethanol at room temperature. 8 weeks was considered to be the maximum length of time that collected *C. sordidus* would remain stored at room temperature prior to DNA extraction since they had to be collected in the field and then shipped in absolute ethanol to ICIPE.

DNA was diluted (1:1000) and the concentration measured by examining its optical density at 260 nm (fig. 6). DNA quality was assessed on a 0.8% agarose gel using

TBE as the running buffer. The yield of DNA from weevils stored for 0, 2, 4, 6 and 8 weeks in 100% ethanol at room temperature decreased significantly between 0 and 2 weeks in storage (fig. 6), ANOVA, F= 12.47, P< 0.001; Tukey multiple range test, P< 0.05). However, from 2 weeks onwards the change in yield did not alter significantly (P< 0.05) and remained at approximately 340 ng DNA/μl in TE buffer (fig. 6). The quality of DNA did not appear to be affected by storage in ethanol at room temperature for up to 8 weeks. In consequence, ethanol preservation was used for all field - collected banana weevils, prior to DNA extraction. Attempts to extract DNA from individuals preserved in lower concentrations of alcohol proved unsatisfactory.

3.1.2. RAPD – PCR analysis

3.1.3. Reproducibility of the method

All studies done to date have found that bands amplified by arbitrarily primed PCR vary in intensity (Black, 1993). Faint bands have been found to be sporadically amplified (Black, 1993). In this study only intensely and consistently amplified bands were included in RAPD dataset. These were identified by performing replicate reactions in a single run on DNA extracted from the same individual and then repeating this over several consecutive runs. However, the oversensitivity of those primers which identified so much variation between closely related *Cosmopolites sordidus* that they were of no use for broader numerical studies was of particular concern. Each unusable primer reduced the number of available markers. In view, however, of the high degeree of variability associated with the extremely A+T rich non-conserved, repetitive regions found in the genome of many insects (Meyer, 1994), it is expected that primers annealing in or around such regions will produce a large number of possibly similar sized amplification products of uncertain homology. The use of such primers is thus to be avoided and a broad preliminary screening of primers is essential for each new taxonomic group to be studied. In the study of genetic variation in the mosquito (Ballinger-Crabtree *et al.*, 1992), forty primers (kits A and B) were tested in the primary screening using two mosquito populations. Four of these were used to test all eleven populations and only three were selected for the analysis. In this study, forty primers (kits A and B) were

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screened. Only five primers out of forty gave consistent results and hence were used for studying genetic variability. Consequently, the genetic variation described in this study, is and should be regarded as, intraspecific particularly in view of findings of Black (1993) and Smith *et al.*, (1994) concerning the co-migration of non-homologous fragments. Homology is established with isozymes by the fact that enzymes from two species react in a common histochemical stain. Homology is assumed with Southern analysis by the fact that two target DNAs hybridize to a common probe. However, with arbitrarily amplified loci there is no reason to believe that bands that comigrate in two species represent homologous regions. Comigrating bands may have no sequence similarity. Furthermore, homologous regions might not be amplified in both of the species to be compared and, even if they are, might vary greatly in size.

Forty random primers (Operon10 mer kit O, A) were screened, five produced discrete scorable bands and did not give rise to amplification products in negative control. Thus, of the 40 primers screened 4 did not produce any amplification products, 19 produced non variable patterns among individuals, 12 produced complex or poorly resolved banding patterns, and 5 produced clear-cut easy to score variable band patterns. The 5 polymorphic O-A primers were used to analyze variation in individuals from each population (table 5). Each variable band was scored as present (1) or absent (0) and was considered to represent the phenotype of a distinct locus. Negative controls were run with each primer to ensure that scorable fragments were not artifacts. The five random primers chosen for analysis generated 46 bands that ranged in size from 100 – 2600 bp. Each primer had between 9 – 10 bands and the grand average for the 15 populations was 9.2 (table 5). RAPD profiles for the five chosen primers were reproducible in terms of presence/absence both within run and between runs. Although there were differences in staining intensity of RAPD fragments between profiles, this was not scored as real variation (fig. 7 A, B, C, D, E) which is an example of RAPD results on a gel after staining with ethidium bromide.

Figure 5:

Showing yield and quality of DNA extracted from banana weevil from four different regions analysed using 0.8% (TBE) agarose gel visualized using ethidium bromide. 1 – Cameroon, 2 – Uganda, 3 – Kenya, 4 – Australia and M – Molecular weight marker.
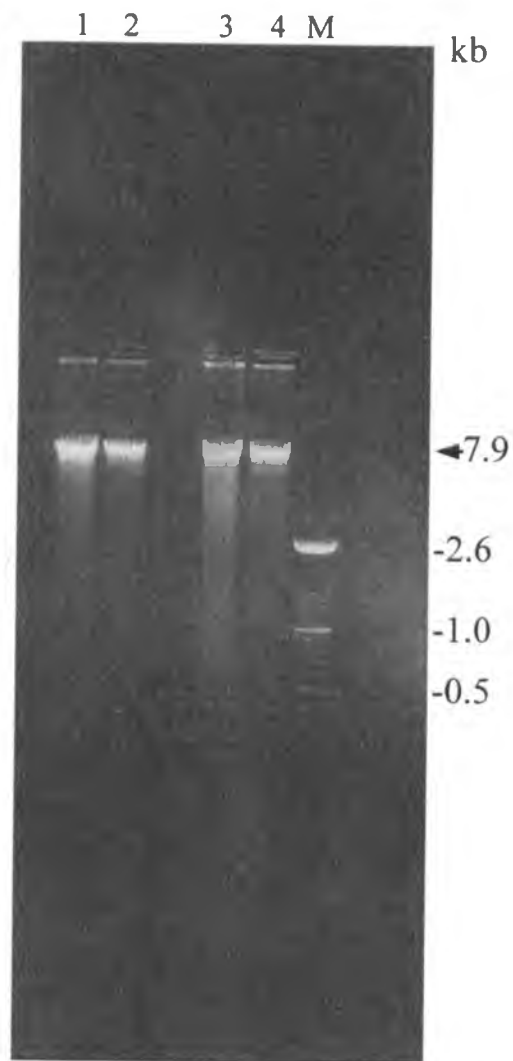
Figure 6:

DNA yield (total nucleic acid) from field collected banana weevils at 0,2,4,6 and 8 weeks of storage in absolute ethanol at room temperature.

Change in DNA concentration with time
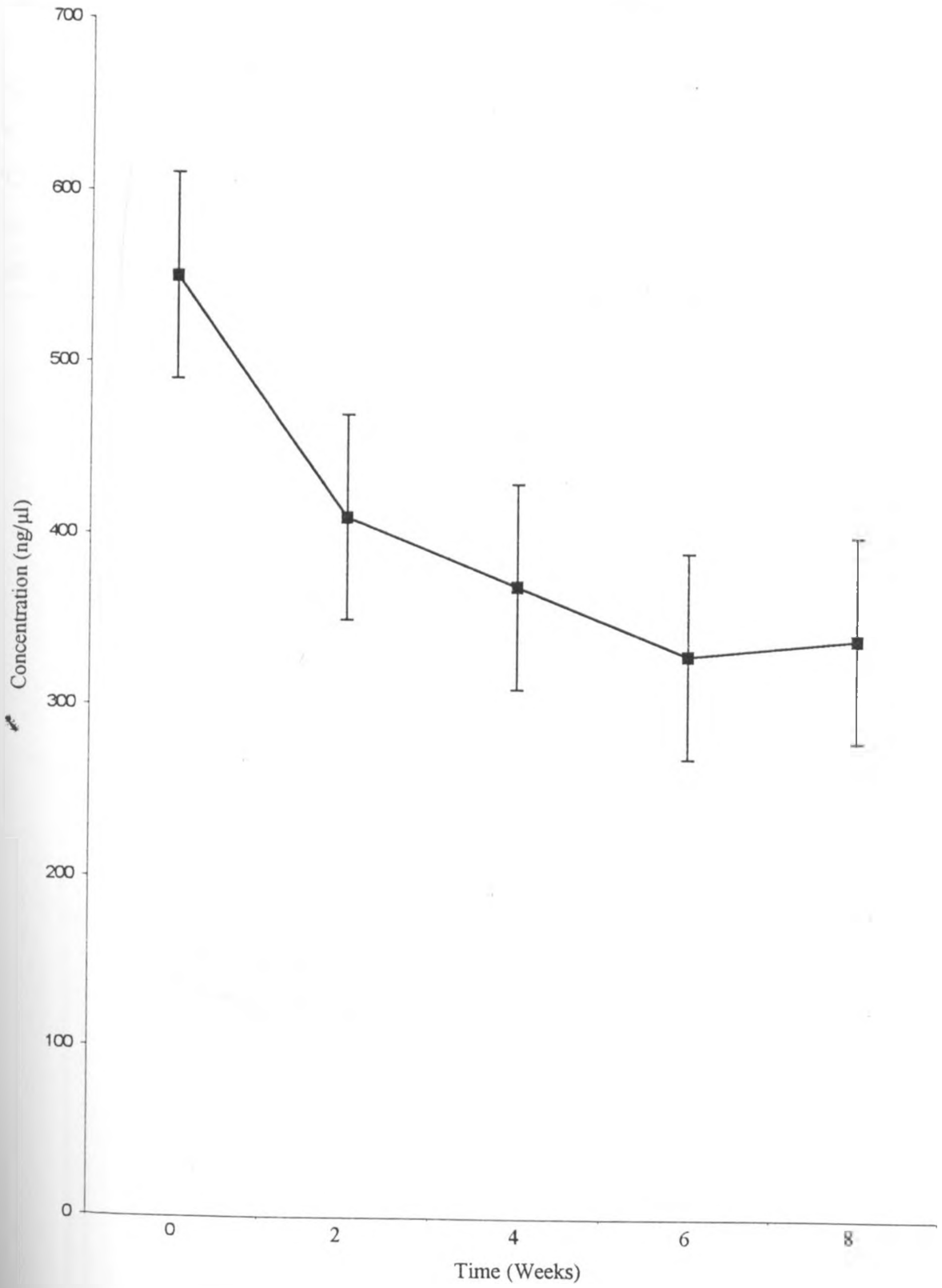
Table 5. Base pair sequence of RAPD primers used

Primer	Base-pair sequence (5' – 3')	Number of RAPD Loci Screened
OPO – 14	AGCATGGCTC	9
OPO – 18	CTCGCTATCC	9
OPO – 19	GGTGCACGTT	10
OPO – 20	ACACACGCTG	9
OPA – 17	GACCGCTTGT	9
Mean of all five primers		9.2

Figure 7:

Example of RAPD polymorphism detected in individuals of *C. sordidus*.
The PCR products were separated on a 1.2% agarose gel, stained with
ethidium bromide.

A – Tanzania samples 1 - 29 amplified using OPO14

B – Tanzania samples 1 – 29 amplified using OPA17

C – Tanzania samples 1 – 29 amplified using OPO19

D – Embu samples 1 – 35 amplified using OPO18

E – Embu samples 1 – 35 amplified using OPO20

B on the left corner of each gel – negative control

M – Molecular weight marker

A

B 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29M

kb
-2.6
-1.5
-1.0

-0.5

-0.1

B

B 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 M

kb
-2.6
-1.5
-1.0

-0.5

-0.1

D kb   M B 1   3   5   7   9   11   13   15   17   19   21   23   25   27   29   31   33   35

2.6-
1.5-
1.0-
0.5-
0.1-

E kb   M B 1   3   5   7   9   11   13   15   17   19   21   23   25   27   29   31   33   35

2.6-
1.5-
1.0-
0.5-
0.1-
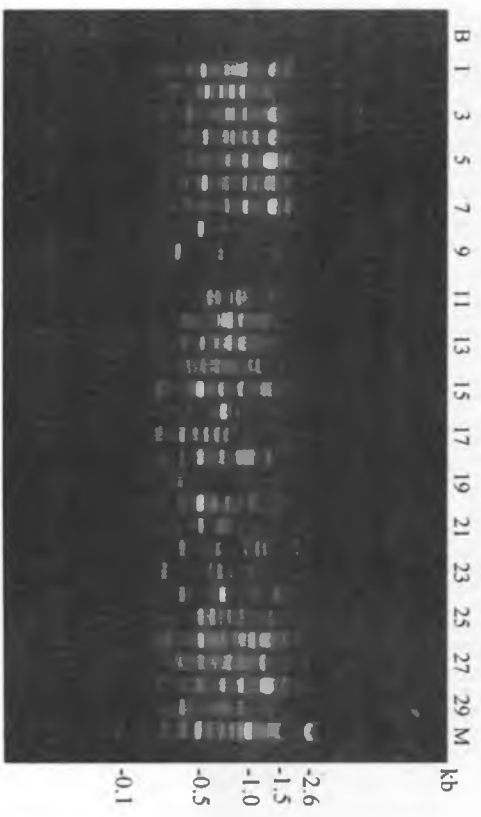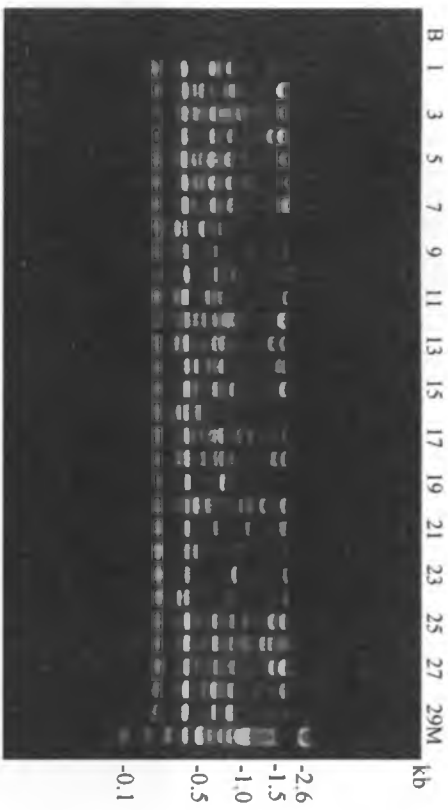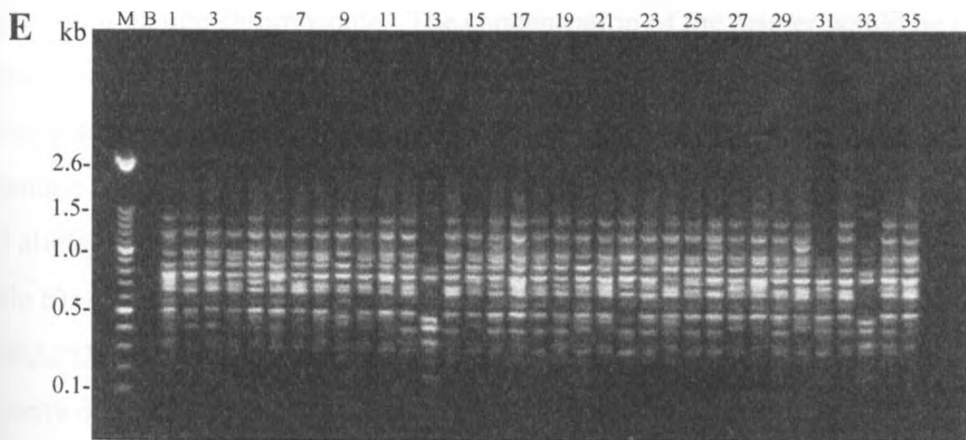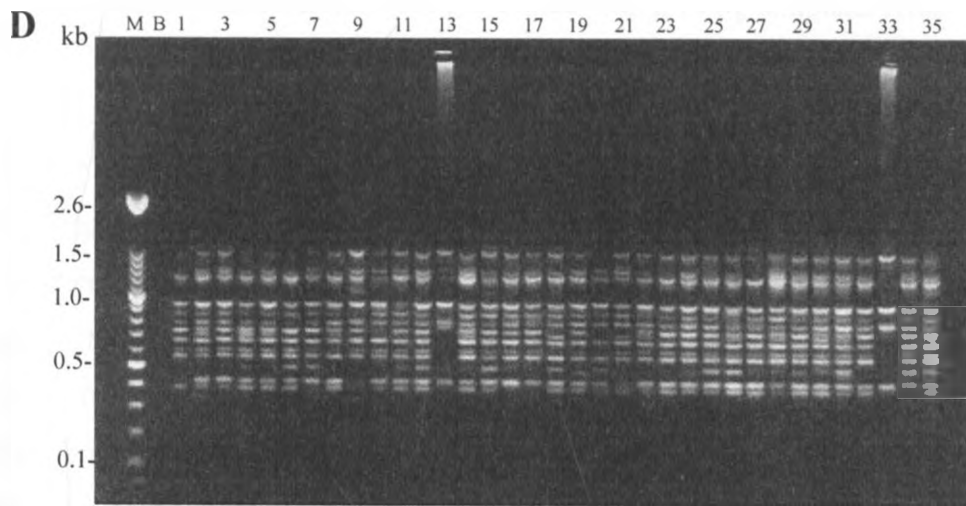
The reproducibility of results between *C. sordidus* tissues (head and legs) was verified by RAPD analysis of DNA extracted separately from these two tissues. DNA from Mbita population was analyzed using the five OPO-A primers. In all instances RAPD fragments patterns for the two tissue types were identical within individuals. The effect of template concentration on the reproducibility of the amplification pattern was assessed at three concentrations. Amplification became inconsistent at or above 200 ng template DNA per reaction and at or below 2 ng template DNA per reaction. Characterization reactions therefore, were performed with a 10 ng template. After amplification the gels were stained with ethidium bromide. The results of each variable bands scored as present (1) or absent (0) for individual sites and primers are shown in appendix III. Fifteen populations were studied. A total of 46 different RAPD loci were generated using 5 primers (table 5).

In order to test whether the 46 RAPD loci were homogenous among the 15 populations, Chi-square ($\chi2$) and likelihood ratio (G^2) were determined among the populations (Hedrick,1984). The presence and absence of bands produced within a single primer are often assumed to be independent. However, Wilkerson *et al.*, (1993) showed that amplification of certain bands interferes with or precludes the amplification of other bands. This might occur through preferential annealing of the primer to a repetition sequence present on one chromosome. The concentration of the primer would be rapidly reduced early in the PCR process. Chi-square analysis was done to test the independence of pairwise association between bands amplified by the same primer and across primers. Once a sample of individuals in a population had been characterized for variation at any locus the allele frequencies were calculated according to Hardy-Weinberg Equilibrium Law (table 6).

The RAPD band frequencies for each of the world population and G^2-tests, for heterogeneity of band frequencies across populations are given in table 6. 17 bands were polymorphic in each population of which 4 were present at a frequency of 0.100 or less in some populations. The frequencies of 27 out of 46 were heterogenous across populations (59%).

Chi-square analysis tested the independence of pairwise association between bands amplified by the same primer and across primers (Legendre and Legendre, 1983).

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Test for independence between pairs of bands amplified by the same primer and across primers revealed 1% (Australia), 9.5% (Zanzibar) to 28.6% (Indonesia) significant association, average 6.7% across the fifteen populations. Since the number of fragments surveyed is large at 46, the few fragments that might correlate would not affect the validity of the results on population structure of banana weevil. RAPD band frequencies calculated in Hardy – Weinberg proportions are indicated in table 6. The number and percentage of polymorphism detected by the selected five primers for the 15 populations of banana weevils from different parts of the world are indicated in table 7. Polymorphism in RAPD banding patterns among individuals of *C. sordidus* was detected (Figure 7 (A), (B), (C), (D), (E). The number of polymorphic RAPD loci per population ranged from 36 (Honduras) to 45 (Puerto Rico, Tanzania and Mbita (Kenya) (table 7). Among *C. sordidus* world populations, genetic diversity varied considerably (table 7 and 8). For instance the percentage polymorphic loci ranged from 78.3% (Honduras) to 97.8% (Mbita, Puerto Rico and Tanzania) averaging 92.2% for the 15 populations (table 7).

Primer OPO14 was on average polymorphic at 90.3% of the loci whereas OPA17, OPO18, OPO19 and OPO20 were on average polymorphic at 96.3%, 94%, 85.3% and 95.6% of the loci respectively.

The gene diversity (h) within each population (averaged over 46 loci) ranged from 0.297 (Honduras) to 0.455 (Mbita (Kenya)) and the mean genetic diversity over all populations was 0.375 (table 8a). The average heterozygosity for individual weevil populations ranged from as high as 0.46 in Mbita population to as low as 0.30 in Honduras population. Higher total diversity (Ht) were calculated with Tanzania (Ht=0.45), Costa Rica (Ht=0.40) and Zanzibar (Ht=0.397) (table 8a). The effect of allele frequency divergence between the different populations on the partitioning of genetic diversity were quantified by the gene diversity statistics of Nei (1973). The total genetic diversity (Ht) was 0.470 over all the populations (table 8b). Within population diversity (Hs) was 0.370 for all loci.

Table 6: RAPD band frequencies in fifteen populations[a] of weevils from different parts of the world calculated using Hardy-Weinberg.

[a]2A = Australia, CA= Cameroon, COL= Colombia, CR= Costa Rica,
Fl= Florida, GH= Ghana, Ho= Honduras, IN= Indonesia, KMB= Kenya (Mbita),
KE=Kenya (Embu), Puerto= Puerto Rico, SA=SouthAfrica, TZ= Tanzania, U1= Uganda,
ZA= Zanzibar
[b]G^2 – test for heterogeneity of band frequencies across populations : *, ** significant at p≤0.05 and 0.0001, respectively.

PRIMER	2A	CA	COL	CR	FL	GH	HO	IN	KMB	KE	PUE	SA	TZ	U1	ZA	G^{2b}
O14-01	0.929	0.820	0.793	0.953	0.583	0.720	1.000	0.786	0.800	0.886	0.692	1.000	0.800	0.960	1.000	22.8**
O14-02	0.643	0.880	1.000	0.953	0.708	0.980	1.000	0.762	0.829	0.971	0.821	1.000	0.860	0.960	1.000	40.68**
O14-03	0.071	0.620	0.724	1.000	0.375	0.940	0.950	0.548	0.771	0.914	0.462	0.349	0.900	0.320	0.708	0.45
O14-04	0.429	0.820	0.897	0.907	0.208	0.960	0.775	0.643	0.657	0.743	0.359	0.442	0.780	0.580	0.813	0.00
O14-05	0.857	0.820	0.931	0.884	0.208	0.920	1.000	0.548	0.800	0.914	0.590	0.953	0.700	0.800	0.625	6.18*
O14-06	0.857	0.860	0.931	1.000	0.833	0.660	1.000	0.571	0.800	0.914	0.615	1.000	0.840	0.860	0.729	15.33**
O14-07	0.214	0.280	0.276	0.977	0.167	0.780	0.675	0.167	0.629	0.971	0.385	0.512	0.860	0.600	0.271	22.47**
O14-08	0.286	0.260	0.000	0.744	0.750	0.780	0.725	0.024	0.629	0.857	0.385	0.535	0.720	0.380	0.333	17.01**
O14-09	0.571	0.800	0.069	0.767	0.125	0.820	0.900	0.048	0.629	0.857	0.256	0.512	0.760	0.200	0.292	112.81**
A17-01	0.714	0.520	0.345	0.419	0.792	0.320	0.075	0.738	0.800	0.400	0.769	0.256	0.680	0.960	0.771	16.82**
A17-02	0.214	0.960	0.828	0.767	0.417	0.900	0.375	0.667	0.743	0.771	0.410	0.721	0.700	0.980	0.688	0.27
A17-03	0.357	0.460	0.828	0.698	0.417	0.960	0.925	0.738	0.857	0.800	0.590	0.721	0.780	0.980	0.813	3.11
A17-04	0.786	0.960	0.966	0.814	0.875	0.940	1.000	0.714	0.771	0.914	0.256	0.837	0.800	0.980	0.833	17.33*
A17-05	0.786	0.920	0.828	0.930	0.833	0.940	1.000	0.690	0.771	0.914	0.359	0.930	0.680	0.980	0.479	10.56*
A17-06	0.786	0.960	0.655	0.791	0.333	0.960	0.875	0.286	0.571	0.886	0.256	0.791	0.800	0.740	0.625	0.04
A17-07	0.929	0.700	0.621	0.907	0.375	0.900	0.975	0.619	0.714	0.886	0.051	0.930	0.540	0.980	0.583	0.46
A17-08	0.286	0.960	0.414	0.953	0.625	0.920	0.925	0.024	0.029	0.829	0.128	0.930	0.580	0.700	0.250	9.25*
A17-09	0.071	0.840	0.207	0.907	0.583	0.880	0.700	0.000	0.000	0.857	0.154	0.837	0.380	0.780	0.000	109.17**
O18-01	0.500	0.680	0.897	0.116	0.375	0.900	0.000	0.667	0.886	0.829	1.000	0.395	0.760	0.800	0.438	0.01
O18-02	0.929	0.440	0.724	0.419	0.792	0.920	0.600	0.714	0.743	0.886	0.949	0.512	0.860	0.920	0.854	3.34
O18-03	0.714	0.980	0.931	0.814	0.750	0.900	0.900	0.405	0.914	0.943	0.923	0.512	0.760	0.920	0.458	10.39*
O18-04	0.714	0.960	0.966	0.907	0.667	0.900	0.950	0.238	0.829	0.943	0.744	0.791	0.760	0.900	0.792	15.53**
O18-05	0.357	0.960	0.828	0.930	0.292	0.860	0.875	0.167	0.800	0.971	0.744	0.465	0.740	0.900	0.583	1.85
O18-06	0.214	1.000	0.724	0.884	0.042	0.820	0.875	0.190	0.457	0.886	0.436	0.442	0.380	0.420	0.250	14.66**
O18-07	0.071	0.580	0.552	0.860	0.583	0.820	0.975	0.048	0.486	1.000	0.692	0.721	0.000	0.860	0.708	0.04
O18-08	0.143	0.940	0.552	0.744	0.208	0.880	0.875	0.000	0.829	0.971	0.487	0.698	0.720	0.920	0.563	0.41
O18-09	0.286	0.520	0.000	0.721	0.292	0.780	0.675	0.000	0.200	0.886	0.256	0.302	0.560	0.860	0.271	118.54**
O19-01	0.143	0.560	0.517	0.093	0.167	0.040	0.000	0.690	0.571	1.000	0.333	0.186	0.200	0.560	0.583	74.92**
O19-02	0.429	0.640	0.690	0.744	0.833	0.920	0.675	0.643	0.514	0.857	0.897	0.442	0.680	0.600	0.771	0.13
O19-03	0.357	0.880	0.828	0.930	0.333	1.000	0.925	0.452	0.686	0.857	0.718	0.907	0.500	1.000	0.771	4.13*
O19-04	0.786	0.980	0.655	0.884	0.583	1.000	0.925	0.357	0.657	0.886	0.821	0.884	0.840	0.940	0.563	7.23*
O19-05	0.000	0.880	0.759	0.907	0.333	1.000	0.975	0.357	0.743	0.886	0.872	0.977	0.860	0.880	0.771	10.12*
O19-06	1.000	0.960	0.828	1.000	0.667	1.000	0.975	0.500	0.771	1.000	0.897	0.907	0.900	0.960	0.896	29.00**
O19-07	0.786	0.960	0.793	0.930	0.917	0.700	1.000	0.238	0.743	0.514	0.821	0.953	0.860	0.980	0.521	4.77*
O19-08	0.643	0.020	0.724	0.767	0.708	0.980	0.900	0.310	0.771	1.000	0.872	0.884	0.900	1.000	0.750	1.49
O19-09	0.786	0.000	0.724	0.767	0.500	0.980	1.000	0.262	0.771	1.000	0.462	0.744	0.760	0.980	0.375	0.24
O19-10	0.357	0.000	0.586	0.140	0.542	0.860	0.900	0.048	0.514	1.000	0.282	0.419	0.920	0.960	0.354	109.32**
O20-01	0.143	0.280	1.000	0.581	0.375	0.900	0.975	0.833	0.743	0.971	0.795	0.744	0.900	0.900	0.833	0.00
O20-02	0.714	0.740	0.724	0.674	0.958	0.980	0.950	0.667	0.800	0.657	0.872	0.977	0.900	0.840	0.896	3.92*
O20-03	0.929	0.860	0.793	0.791	0.917	1.000	0.975	0.714	0.829	0.886	0.872	0.907	0.860	0.920	0.833	9.96*

O20-04	0.857	0.860	0.793	0.814	0.625	1.000	0.975	0.714	0.886	0.943	0.667	0.907	0.900	0.940	0.958	9.35*
O20-05	0.786	0.780	0.690	0.814	0.500	0.980	0.975	0.452	0.886	1.000	0.667	0.884	0.880	0.940	0.792	2.36
O20-06	0.714	0.820	0.621	0.767	0.667	0.960	0.975	0.381	0.886	0.971	0.436	0.884	0.880	0.940	0.729	0.55
O20-07	0.786	0.800	0.552	0.721	0.750	1.000	0.850	0.333	0.829	0.971	0.256	0.907	0.880	0.920	0.750	0.00
O20-08	0.714	0.840	0.241	0.581	0.792	0.960	0.925	0.214	0.829	0.914	0.256	0.651	0.840	0.940	0.667	3.08
O20-09	0.071	0.360	0.000	0.512	0.542	0.900	0.650	0.119	0.800	0.886	0.077	0.791	0.600	0.880	0.042	118.29**

Table 7: Number and percentage of polymorphism detected by the selected five primers for 15 populations[a] of banana weevils from different parts of the world.

[a]2A = Australia, CA= Cameroon, COL= Colombia, CR= Costa Rica, Fl= Florida, GH= Ghana, Ho= Honduras, IN= Indonesia, KMB= Kenya (Mbita), KE=Kenya (Embu), Puerto= Puerto Rico, SA=SouthAfrica, TZ= Tanzania, U1= Uganda, ZA= Zanzibar

PRIMER	2A	CA	COL	CR	FL	GH	HO	IN	KMB	KE	PUE	SA	TZ	U1	ZA	MEAN
OPO14	9	9	7	7	9	9	5	9	9	9	9	6	9	9	7	8.1
%	100	100	77.8	77.8	100	100	55.5	100	100	100	100	66.7	100	100	77.8	90.3
OPA17	9	9	9	9	9	9	7	8	8	9	9	9	9	9	8	8.7
%	100	100	100	100	100	100	77.8	88.9	88.9	100	100	100	100	100	88.9	96.3
OPO18	9	8	8	9	9	9	8	7	9	8	8	9	8	9	9	8.5
%	100	88.9	88.9	100	100	100	88.9	77.8	100	88.9	88.9	100	88.9	100	100	94.0
OPO19	8	8	10	9	6	7	7	10	10	5	10	10	10	8	10	8.5
%	80	80	100	90	60	70	70	100	100	50	100	100	100	80	80	85.3
OPO20	9	9	7	9	9	6	9	9	9	8	9	9	9	9	9	8.6
%	100	100	77.8	100	100	66.7	100	100	100	88.9	100	100	100	100	100	95.6
TOTAL	44	43	41	43	42	40	36	43	45	39	45	43	45	44	43	42.4
MEAN	95.6	93.5	89.1	93.5	91.3	86.9	78.3	93.5	97.8	84.8	97.8	93.5	97.8	95.6	93.5	92.2

Estimates of Shannon's phenotypic diversity (H_o) for RAPD's are given in table 8 (King and Schaal, 1989). They ranged from 0.382 (Honduras) to 0.806 (Florida) and the mean for the 15 populations was 0.623. Estimates of H_o also varied among primers with OPO19 detecting the lowest 0.597 and OPO18 detecting the highest 0.647 value of diversity (table 9). Shannon's information index of phenotypic diversity was used to partition the diversity into within - and between population components (table 10). H_{pop} provided a measure of the average diversity of the fifteen populations. OPO18 detected the most and OPO19 the least within- population variability. A t-test was carried out to evaluate differences between genetic distances within and between *C. sordidus* populations. The statistical result showed that the variation within and between *C. sordidus* populations was significantly different (p<0.0001). An examination of genetic diversity present within populations (H_{pop}/H_{sp}), and between populations, ($H_{sp} - H_{pop})/H_{sp}$, indicated that on the average the diversity (74.7%) mainly occurred within *C. sordidus*

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populations. The distribution of variability between - and within - populations, however, varied between primers. For example, OPO19 detected most variability between populations (27.5%), whereas primer OPO20 and OPO14 detected most variation within populations (78.7% and 76.6%), respectively (Table 9). The genetic diversity within and among the 15 populations revealed a high level of divergence (Gst value of 0.216) (table 9). This means that approximately 22% of the genetic variation observed in this study was due to differentiation among populations of *C. sordidus* compared with 78% within populations. Estimates of gene flow (Nm) (table 17) ranged from 0.617 to 1.05. Over all populations, according to Crow and Aoki (1984), gene flow values (Nm) of less than 1 should be interpreted as little or no gene flow. Thus, the average of the values (Nm=0.811) observed within populations in this study would indicate extremely rare migratory events.

Table 8a: Average Heterozygousity and its Standard error calculated for Banana Weevil Populations from different parts of the world.

Population Number	Country	Average heterozygousity	Standard Error
1	Australia	0.357481	0.024852
2	Cameroon	0.364895	0.020914
3	Columbia	0.383447	0.023916
4	Costa Rica	0.401675	0.021749
5	Florida	0.387155	0.019130
6	Ghana	0.333383	0.025293
7	Honduras	0.297153	0.027561
8	Indonesia	0.324004	0.026621
9	Kenya (Mbita)	0.455424	0.015939
10	Kenya (Embu)	0.345164	0.024985
11	Puerto Rico	0.372587	0.019660
12	South Africa	0.384329	0.019027
13	Tanzania	0.446906	0.013511
14	Uganda	0.367122	0.017507
15	Zanzibar	0.397390	0.021764

Table 8b:

Partitioning of total diversity (Ht) for each polymorphic locus and averaged for the 46 loci: diversity within population (Hs), between populations gene diversity (Dst), and proportion of total allelic diversity found among populations (Gst).

Locus number	Gst	Ht	Hs	Dst
1	0.280109	0.468080	0.336967	0.131113
2	0.212479	0.385439	0.303541	0.081898
3	0.282031	0.497437	0.357144	0.140293
4	0.147433	0.496081	0.422942	0.073139
5	0.182499	0.490293	0.400815	0.089478
6	0.169919	0.447986	0.371865	0.076121
7	0.282882	0.453833	0.325451	0.128382
8	0.146126	0.437153	0.373274	0.063879
9	0.223825	0.413860	0.321228	0.092632
10	0.169078	0.468817	0.389550	0.079267
11	0.169959	0.498094	0.413439	0.084655
12	0.143196	0.499600	0.428060	0.071540
13	0.166458	0.462535	0.385543	0.076992
14	0.178345	0.477330	0.392200	0.085130
15	0.167898	0.499534	0.415663	0.083871
16	0.224407	0.499162	0.387147	0.111988
17	0.305942	0.475535	0.330049	0.145486
18	0.330446	0.455479	0.304967	0.150512
19	0.274905	0.492246	0.356925	0.135321
20	0.132023	0.492542	0.427515	0.065027
21	0.147237	0.486986	0.415283	0.071703
22	0.131201	0.483944	0.420450	0.063494
23	0.208435	0.499998	0.395781	0.104217
24	0.307744	0.466787	0.323136	0.143651
25	0.335385	0.485550	0.322704	0.162846
26	0.271211	0.491376	0.358109	0.133267
27	0.217507	0.405126	0.317009	0.088117
28	0.319147	0.375100	0.255388	0.119712
29	0.109445	0.491004	0.437266	0.053738
30	0.252971	0.493101	0.368361	0.124740
31	0.190014	0.481026	0.389625	0.091401
32	0.271499	0.491341	0.357942	0.133399
33	0.229209	0.391111	0.301465	0.089646
34	0.221307	0.483146	0.376222	0.106924
35	0.282149	0.489766	0.351579	0.138187
36	0.351971	0.499706	0.323824	0.175882
37	0.379681	0.470943	0.292135	0.178808
38	0.261287	0.495302	0.365886	0.129416
39	0.113147	0.464444	0.411894	0.05255

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40	0.071540	0.445021	0.413184	0.031837
41	0.111771	0.451286	0.400845	0.050441
42	0.153090	0.488710	0.413893	0.074817
43	0.152839	0.490806	0.415792	0.075014
44	0.194788	0.492688	0.396718	0.095970
45	0.195533	0.499792	0.402066	0.097726
46	0.290402	0.442291	0.313849	0.128442
All loci	0.216117	0.471030	0.369232	0.101798

Table 9: Estimates of Shannon's phenotypic diversity index for RAPD's (H_o) for fifteen populations[a] of Banana weevil from different parts of the world.

[a]2A = Australia, CA= Cameroon, COL= Colombia, CR= Costa Rica,
Fl= Florida, GH= Ghana, Ho= Honduras, IN= Indonesia, KMB= Kenya (Mbita),
KE=Kenya (Embu), Puerto= Puerto Rico, SA=SouthAfrica, TZ= Tanzania, U1=
Uganda, ZA= Zanzibar

PRIMER	2A	CA	COL	CR	FL	GH	HO	IN	KMB	KE	PUE	SA	TZ	U1	ZA	MEAN
OPO14	0.716	0.724	0.445	0.363	0.771	0.566	0.365	0.727	0.821	0.462	0.910	0.577	0.698	0.703	0.588	0.630
OPA17	0.712	0.542	0.748	0.623	0.855	0.435	0.411	0.702	0.636	0.621	0.753	0.627	0.870	0.380	0.751	0.644
OPO18	0.735	0.536	0.601	0.642	0.792	0.507	0.493	0.575	0.724	0.356	0.669	0.924	0.733	0.557	0.868	0.647
OPO19	0.665	0.376	0.836	0.511	0.832	0.239	0.300	0.850	0.878	0.321	0.705	0.585	0.656	0.359	0.848	0.597
OPO20	0.668	0.732	0.662	0.848	0.782	0.190	0.340	0.827	0.643	0.361	0.744	0.559	0.576	0.416	0.621	0.598
MEAN	0.699	0.582	0.658	0.597	0.806	0.387	0.382	0.736	0.740	0..424	0.756	0.654	0.707	0.483	0.735	0.623

Table 10: Components of Genetic diversity in *C. sordidus* from 15 locations from different parts of the world partitioned into within and between populations for 5 random oligonucleotide primers. H_{pop}=Average diversity of RAPD markers for all populations, H_{sp} = Shannon's within species diversity index, H_{pop}/H_{sp} = within population diversity, $(H_{sp}-H_{pop})/H_{sp}$= diversity among populations.

Primer	H_{pop}	H_{sp}	H_{pop}/H_{sp}	$(H_{sp}-H_{pop})/H_{sp}$
OPO14	0.630	0.822	0.766	0.234
OPA17	0.644	0.873	0.738	0.262
OPO18	0.647	0.891	0.726	0.274
OPO19	0.597	0.824	0.725	0.275
OPO20	0.598	0.760	0.787	0.213
Mean	0.623	0.834	0.747	0.253

3.2. Genetic diversity within and among Banana Weevil populations from different regions in Uganda using RAPD.

PCR products were run on gels and the gels stained with ethidium bromide. RAPDs were scored as present (1) or absent (0) and the results are indicated in appendix IV. RAPD band frequencies in fifteen populations of banana weevils from different regions in Uganda were calculated according to Hardy-Weinberg as indicated in section 1.9.4 and the results are indicated in table 13. Chi-square ($\chi2$) and likelihood ratio (G^2) were performed among the populations to test whether the 37 RAPD loci were homogenous among the 15 populations.

The RAPD band frequencies for each of the Uganda populations and G^2-test for heterogeneity of band frequencies across populations are given in table 13. 16 bands were polymorphic in each population of which 2 were present at a frequency of 0.100 or less in some populations. The frequencies of 18 out of 37 were heterogenous across populations (48.6%). Therefore, RAPD divergence among natural populations of banana weevil was due mainly to band frequency differences rather than the fixation of locally common or rare bands (table 11).

Test for independence between pairs of band amplified by the same primer and across primers using Chi-square analysis revealed 2.74% (Uganda 2) to 12.9% (Uganda 15) significant association, average 6.7% across the fifteen populations. Since the number of fragments surveryed is large at 37, the few fragments that might correlate would not affect the validity of the results on populations structure of the banana weevil. A total of 37 different RAPD loci were generated using 4 primers. The number of polymorphic RAPD loci per population ranged from 29 (Buwama) to 37 (Kalegweri, Namubiru, Ntungamo and Bundagara) (table 12). Among the Uganda populations there was some degree of variation (table 12, 14). The percentage polymorphic loci ranged from78% (Buwama) to 100% (Kalegweri, Namubiru, Ntungamo and Bundagara) averaging 94.2% for the 15 populations.

Primer OPA17 was on average polymorphic at 94.7% of the loci whereas OPA17, OPO18, OPO19 and OPO20 were on average polymorphic at 87.4%, 94.7%, 85.3% and 94% of the loci respectively (table 12).

The gene diversity (h) within each population (averaged over 37 loci) ranged from 0.321 (Ntengeru) to 0.468 (Namubiru) and the mean genetic diversity over all populations was 0.408 (table 13a). The mean estimated heteroygousity for individual populations ranged from 0.47 for Namubiru to 0.32 for Ntengeru (table 13a). The total genetic diversity (Ht) was 0.46 at all loci. Within poulation diversity (Hs) was 0.40. Between population diversity (Dst) was only 0.060405, therefore within population diversity (Hs) was responsible for most of the genetic diversity (table 13b).

Table 11 : RAPD Band frequencies in fifteen populations[a] of banana weevils from different regions in Uganda calculated using Hardy-Weinberg.

[a]UG2= Kalegweri, UG3= Ikulwe, UG4= Ntengeru, UG5= Buwama, UG6= Bukanga, UG7= Buzaya, UG8= Lakeshores, UG9= Bunyaruguru, UG10= Namubiru, UG11= Ntungamo, UG12= Kabukye, UG13= Bundagara, UG14= Namunonyi, UG15= Kawanda, UG16= Ntinkalu.

[b]G^2 – test for heterogeneity of band frequency across populations : *, ** significant at $p \leq 0.05$ and 0.0001, respectively.

PRIMER	UG 2	UG 3	UG 4	UG 5	UG 6	UG 7	UG 8	UG 9	UG 10	UG 11	UG 12	UG 13	UG 14	UG 15	UG 16	G^{2b}
OPA17-01	0.650	0.966	0.793	1.000	0.759	1.000	0.931	0.828	0.690	0.690	0.966	0.931	0.759	0.345	0.655	6.60*
OPA17-02	0.750	0.966	0.793	0.793	0.448	0.724	0.931	0.724	0.621	0.655	0.793	0.828	0.724	0.586	0.655	0.80
OPA17-03	0.850	0.966	0.828	1.000	0.793	0.966	0.931	0.759	0.655	0.897	0.931	0.828	0.690	0.724	0.862	14.50**
OPA17-04	0.850	0.966	0.862	1.000	0.862	1.000	0.931	0.862	0.690	0.966	0.793	0.897	0.724	0.414	0.552	10.75*
OPA17-05	0.900	0.966	0.897	0.793	0.931	1.000	0.966	0.724	0.690	0.966	0.759	0.759	0.862	0.862	0.897	18.29**
OPA17-06	0.800	0.862	0.759	0.966	0.931	0.862	0.828	0.759	0.621	0.931	0.414	0.862	0.034	0.586	0.586	0.28
OPA17-07	0.850	0.966	0.897	0.966	0.931	1.000	0.966	0.759	0.690	0.966	0.655	0.862	0.793	0.828	0.828	18.29**
OPA17-08	0.550	0.586	0.034	0.345	0.621	0.483	0.552	0.517	0.483	0.448	0.759	0.690	0.241	0.448	0.690	29.38**
OPA17-09	0.500	0.690	0.379	0.034	0.276	0.483	0.655	0.138	0.276	0.690	0.000	0.276	0.207	0.276	0.241	5.25*
OPO18-01	0.900	0.828	0.966	0.517	0.931	0.966	0.828	0.931	0.862	0.793	0.897	0.966	0.966	0.897	0.931	5.82*
OPO18-02	0.750	0.931	0.966	1.000	0.828	0.931	0.931	0.759	0.931	0.862	0.931	0.966	0.690	0.897	1.000	7.57*
OPO18-03	0.750	0.966	0.862	1.000	0.793	0.931	0.966	0.828	0.862	0.655	0.862	0.897	0.448	0.931	0.931	2.63
OPO18-04	0.800	1.000	0.966	1.000	0.897	0.966	1.000	0.897	0.862	0.897	0.931	0.897	0.862	0.897	0.966	12.12*
OPO18-05	0.750	0.966	0.759	0.862	0.759	0.759	0.966	0.621	0.828	0.552	0.931	0.759	0.690	0.793	0.241	0.62
OPO18-06	0.700	0.931	0.862	0.724	0.724	0.897	0.931	0.862	0.862	0.862	0.828	0.931	0.931	0.690	0.586	0.99
OPO18-07	0.150	0.793	0.690	0.448	0.724	0.828	0.828	1.000	0.690	0.379	0.172	0.379	0.586	0.621	0.552	23.70**
OPO18-08	0.750	0.724	0.828	0.966	0.828	0.897	0.724	0.862	0.862	0.862	0.828	0.966	0.655	0.793	0.931	1.61
OPO18-09	0.500	0.828	0.862	0.000	0.517	0.690	0.793	0.483	0.621	0.759	0.724	0.897	0.310	0.069	0.138	0.38
OPO19-01	0.900	0.759	0.103	0.966	0.724	0.931	0.759	0.414	0.828	0.793	0.793	0.448	0.931	0.759	0.828	1.34
OPO19-02	0.950	0.793	1.000	0.966	0.897	0.966	0.793	0.310	0.862	0.552	0.931	0.862	0.897	0.759	0.586	0.63
OPO19-03	0.950	0.724	1.000	0.966	0.931	1.000	0.759	0.621	0.828	0.966	0.862	0.966	0.897	0.897	0.862	7.09*
OPO19-04	0.950	0.621	1.000	0.966	0.897	0.931	0.655	0.586	0.862	0.931	0.552	0.828	0.897	0.793	0.724	0.93
OPO19-05	0.900	0.552	0.517	0.897	0.897	0.276	0.552	0.724	0.862	0.621	0.586	0.414	0.310	0.621	0.517	16.02**
OPO19-06	0.950	0.793	1.000	0.862	0.931	0.966	0.793	0.759	0.862	0.966	0.793	0.897	0.897	0.828	0.621	1.61*
OPO19-07	0.900	0.655	0.931	0.690	0.690	0.862	0.690	0.759	0.828	0.897	0.621	0.690	0.897	0.724	0.759	0.00
OPO19-08	0.950	0.793	1.000	0.966	0.897	0.897	0.793	0.690	0.793	0.966	0.793	0.897	0.862	0.862	0.759	4.64*
OPO19-09	0.700	0.759	0.448	0.966	0.828	0.793	0.759	0.621	0.828	0.897	0.379	0.690	0.793	0.724	0.586	2.10
OPO19-10	0.400	0.759	0.828	0.000	0.793	0.931	0.759	0.828	0.759	0.931	0.483	0.414	0.897	0.000	0.586	1.25
OPO20-01	0.750	1.000	0.828	0.103	0.966	0.345	1.000	0.483	0.207	0.483	0.759	0.414	0.897	0.517	0.690	11.54*
OPO20-02	0.900	1.000	1.000	0.621	1.000	0.966	1.000	0.448	0.862	0.966	0.966	0.897	0.931	0.966	0.793	9.68*
OPO20-03	0.900	0.897	1.000	0.621	0.897	0.655	0.897	0.931	0.862	0.931	0.966	0.931	0.897	0.931	0.586	5.90*
OPO20-04	0.650	0.828	0.862	0.586	0.690	0.931	0.862	0.690	0.828	0.828	0.931	0.586	0.759	0.862	0.586	0.00
OPO20-05	0.950	0.897	0.793	0.552	0.931	0.931	0.931	0.793	0.862	0.759	0.862	0.897	0.828	0.759	0.759	3.24
OPO20-06	0.900	0.966	0.862	0.655	0.966	0.586	0.966	0.655	0.862	0.931	0.759	0.897	0.828	0.828	0.897	3.45
OPO20-07	0.950	0.931	0.793	0.655	0.517	0.690	0.931	0.655	0.862	0.931	0.828	0.690	0.862	0.517	0.483	0.05
OPO20-08	0.950	0.897	0.862	0.690	0.621	0.862	0.897	0.655	0.862	0.931	0.828	0.759	0.793	0.621	0.828	1.10
OPO20-09	0.700	0.655	0.241	0.643	0.931	0.621	0.655	0.207	0.862	0.862	0.517	0.448	0.000	0.310	0.172	0.17

Table 11 : RAPD Band frequencies in fifteen populations[a] of banana weevils from different regions in Uganda calculated using Hardy-Weinberg.

[a]UG2= Kalegweri, UG3= Ikulwe, UG4= Ntengeru, UG5= Buwama, UG6= Bukanga, UG7= Buzaya, UG8= Lakeshores, UG9= Bunyaruguru, UG10= Namubiru, UG11= Ntungamo, UG12= Kabukye, UG13= Bundagara, UG14= Namunonyi, UG15= Kawanda, UG16= Ntinkalu.
[b]G^2 – test for heterogeneity of band frequency across populations : *, ** significant at p≤0.05 and 0.0001, respectively.

PRIMER	UG 2	UG 3	UG 4	UG 5	UG 6	UG 7	UG 8	UG 9	UG 10	UG 11	UG 12	UG 13	UG 14	UG 15	UG 16	G^{2b}
OPA17-01	0.650	0.966	0.793	1.000	0.759	1.000	0.931	0.828	0.690	0.690	0.966	0.931	0.759	0.345	0.655	6.60*
OPA17-02	0.750	0.966	0.793	0.793	0.448	0.724	0.931	0.724	0.621	0.655	0.793	0.828	0.724	0.586	0.655	0.80
OPA17-03	0.850	0.966	0.828	1.000	0.793	0.966	0.931	0.759	0.655	0.897	0.931	0.828	0.690	0.724	0.862	14.50**
OPA17-04	0.850	0.966	0.862	1.000	0.862	1.000	0.931	0.862	0.690	0.966	0.793	0.897	0.724	0.414	0.552	10.75*
OPA17-05	0.900	0.966	0.897	0.793	0.931	1.000	0.966	0.724	0.690	0.966	0.759	0.759	0.862	0.862	0.897	18.29**
OPA17-06	0.800	0.862	0.759	0.966	0.931	0.862	0.828	0.759	0.621	0.931	0.414	0.862	0.034	0.586	0.586	0.28
OPA17-07	0.850	0.966	0.897	0.966	0.931	1.000	0.966	0.759	0.690	0.966	0.655	0.862	0.793	0.828	0.828	18.29**
OPA17-08	0.550	0.586	0.034	0.345	0.621	0.483	0.552	0.517	0.483	0.448	0.759	0.690	0.241	0.448	0.690	29.38**
OPA17-09	0.500	0.690	0.379	0.034	0.276	0.483	0.655	0.138	0.276	0.690	0.000	0.276	0.207	0.276	0.241	5.25*
OPO18-01	0.900	0.828	0.966	0.517	0.931	0.966	0.828	0.931	0.862	0.793	0.897	0.966	0.966	0.897	0.931	5.82*
OPO18-02	0.750	0.931	0.966	1.000	0.828	0.931	0.931	0.759	0.931	0.862	0.931	0.966	0.690	0.897	1.000	7.57*
OPO18-03	0.750	0.966	0.862	1.000	0.793	0.931	0.966	0.828	0.862	0.655	0.862	0.897	0.448	0.931	0.931	2.63
OPO18-04	0.800	1.000	0.966	1.000	0.897	0.966	1.000	0.897	0.862	0.897	0.931	0.897	0.862	0.897	0.966	12.12*
OPO18-05	0.750	0.966	0.759	0.862	0.759	0.759	0.966	0.621	0.828	0.552	0.931	0.759	0.690	0.793	0.241	0.62
OPO18-06	0.700	0.931	0.862	0.724	0.724	0.897	0.931	0.862	0.862	0.862	0.828	0.931	0.931	0.690	0.586	0.99
OPO18-07	0.150	0.793	0.690	0.448	0.724	0.828	0.828	1.000	0.690	0.379	0.172	0.379	0.586	0.621	0.552	23.70**
OPO18-08	0.750	0.724	0.828	0.966	0.828	0.897	0.724	0.862	0.862	0.862	0.828	0.966	0.655	0.793	0.931	1.61
OPO18-09	0.500	0.828	0.862	0.000	0.517	0.690	0.793	0.483	0.621	0.759	0.724	0.897	0.310	0.069	0.138	0.38
OPO19-01	0.900	0.759	0.103	0.966	0.724	0.931	0.759	0.414	0.828	0.793	0.793	0.448	0.931	0.759	0.828	1.34
OPO19-02	0.950	0.793	1.000	0.966	0.897	0.966	0.793	0.310	0.862	0.552	0.931	0.862	0.897	0.759	0.586	0.63
OPO19-03	0.950	0.724	1.000	0.966	0.931	1.000	0.759	0.621	0.828	0.966	0.862	0.966	0.897	0.897	0.862	7.09*
OPO19-04	0.950	0.621	1.000	0.966	0.897	0.931	0.655	0.586	0.862	0.931	0.552	0.828	0.897	0.793	0.724	0.93
OPO19-05	0.900	0.552	0.517	0.897	0.897	0.276	0.552	0.724	0.862	0.621	0.586	0.414	0.310	0.621	0.517	16.02**
OPO19-06	0.950	0.793	1.000	0.862	0.931	0.966	0.793	0.759	0.862	0.966	0.793	0.897	0.897	0.828	0.621	1.61*
OPO19-07	0.900	0.655	0.931	0.690	0.690	0.862	0.690	0.759	0.828	0.897	0.621	0.690	0.897	0.724	0.759	0.00
OPO19-08	0.950	0.793	1.000	0.966	0.897	0.897	0.793	0.690	0.793	0.966	0.793	0.897	0.862	0.862	0.759	4.64*
OPO19-09	0.700	0.759	0.448	0.966	0.828	0.793	0.759	0.621	0.828	0.897	0.379	0.690	0.793	0.724	0.586	2.10
OPO19-10	0.400	0.759	0.828	0.000	0.793	0.931	0.759	0.828	0.759	0.931	0.483	0.414	0.897	0.000	0.586	1.25
OPO20-01	0.750	1.000	0.828	0.103	0.966	0.345	1.000	0.483	0.207	0.483	0.759	0.414	0.897	0.517	0.690	11.54*
OPO20-02	0.900	1.000	1.000	0.621	1.000	0.966	1.000	0.448	0.862	0.966	0.966	0.897	0.931	0.966	0.793	9.68*
OPO20-03	0.900	0.897	1.000	0.621	0.897	0.655	0.897	0.931	0.862	0.931	0.966	0.931	0.897	0.931	0.586	5.90*
OPO20-04	0.650	0.828	0.862	0.586	0.690	0.931	0.862	0.690	0.828	0.828	0.931	0.586	0.759	0.862	0.586	0.00
OPO20-05	0.950	0.897	0.793	0.552	0.931	0.931	0.931	0.793	0.862	0.759	0.862	0.897	0.828	0.759	0.759	3.24
OPO20-06	0.900	0.966	0.862	0.655	0.966	0.586	0.966	0.655	0.862	0.931	0.759	0.897	0.828	0.828	0.897	3.45
OPO20-07	0.950	0.931	0.793	0.655	0.517	0.690	0.931	0.655	0.862	0.931	0.828	0.690	0.862	0.517	0.483	0.05
OPO20-08	0.950	0.897	0.862	0.690	0.621	0.862	0.897	0.655	0.862	0.931	0.828	0.759	0.793	0.621	0.828	1.10
OPO20-09	0.700	0.655	0.241	0.643	0.931	0.621	0.655	0.207	0.862	0.862	0.517	0.448	0.000	0.310	0.172	0.17

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Table 12: Number and percentage of polymorphism detected by the selected four primers for fifteen populations[a] of banana weevils from Uganda.

[a]UG2= Kalegweri, UG3= Ikulwe, UG4= Ntengeru, UG5= Buwama, UG6= Bukanga, UG7= Buzaya, UG8= Lakeshores, UG9= Bunyaruguru, UG10= Namubiru, UG11= Ntungamo, UG12= Kabukye, UG13= Bundagara, UG14= Namunonyi, UG15= Kawanda, UG16= Ntinkalu.

PRIMER	UG 2	UG 3	UG 4	UG 5	UG 6	UG 7	UG 8	UG 9	UG 10	UG 11	UG 12	UG 13	UG 14	UG 15	UG 16	MEAN
OPA17	9	9	9	6	9	5	9	9	9	9	8	9	9	9	9	8.5
%	100	100	100	66.6	100	55.5	100	55.5	100	100	88.9	100	100	100	100	94.7
OPO18	9	8	9	5	9	9	8	8	9	9	9	9	9	9	8	8.5
%	100	88.9	100	55.5	100	100	88.9	88.9	100	100	100	100	100	100	88.9	87.4
OPO19	10	10	5	9	10	9	10	10	10	10	10	10	10	9	10	9.5
%	100	100	50	90	100	90	100	100	100	100	100	100	100	90	100	94.7
OPO20	9	7	7	9	8	9	7	9	9	9	9	9	8	9	9	8.5
%	100	77.8	77.8	100	88.9	100	77.8	100	100	100	100	100	88.9	100	100	94.0
TOTAL	37	34	30	29	36	32	34	36	37	37	36	37	36	36	36	34.9
MEAN	100	91.1	81.1	78.0	97.3	86.5	91.9	97.3	100	100	97.3	100	97.3	97.3	97.3	94.2

Table 13a: Average heterozygousity and its standard error (Uganda populations)

Population Number	Site	Average Heterozygousity	Standard Error
1	Kalegweri	0.439824	0.012738
2	Ikulwe	0.401940	0.023520
3	Ntengeru	0.321372	0.033339
4	Buwama	0.331807	0.030293
5	Bukanga	0.416766	0.019513
6	Buzaya	0.362676	0.026511
7	Lakeshores	0.402659	0.023101
8	Bunyaruguru	0.438525	0.018666
9	Namubiru	0.467596	0.010313
10	Ntungamo	0.423712	0.011745
11	Kabukye	0.426844	0.017412
12	Bundagara	0.424135	0.011426
13	Namunyonyi	0.415187	0.020654
14	Kawanda	0.426034	0.019161
15	Ntinkalu	0.421654	0.019819

Table 13b: Partitioning of total diversity (Ht), for each polymorphic locus and averaged for the 37 loci: diversity within population (Hs), between populations gene diversity (Dst), and proportion of total allelic diversity found among populations (Gst).

Locus number	Gst	Ht	Hs	Dst
1	0.211798	0.475859	0.375073	0.100786
2	0.074135	0.499996	0.462929	0.037067
3	0.104294	0.462242	0.414033	0.048209
4	0.192715	0.461912	0.372894	0.089018
5	0.100304	0.446295	0.401530	0.044765
6	0.167374	0.499712	0.416073	0.083639
7	0.112327	0.444755	0.394797	0.049958
8	0.070446	0.420692	0.391056	0.029636
9	0.121597	0.320639	0.281651	0.038988
10	0.079570	0.437295	0.402499	0.034796
11	0.120715	0.411408	0.361744	0.049664
12	0.131148	0.456821	0.396910	0.059911
13	0.106628	0.366183	0.327138	0.039045
14	0.120308	0.498232	0.438290	0.059942
15	0.061227	0.481849	0.452347	0.029502
16	0.210248	0.479893	0.378997	0.100896
17	0.081966	0.489381	0.499268	0.040113
18	0.193586	0.460949	0.371716	0.089233
19	0.157181	0.499313	0.420830	0.078483
20	0.185429	0.476614	0.388236	0.088378
21	0.139228	0.418822	0.360511	0.058311
22	0.149038	0.476873	0.405801	0.071072
23	0.128915	0.482378	0.420192	0.062186
24	0.097506	0.450156	0.406263	0.043893
25	0.049699	0.493532	0.469004	0.024528
26	0.089069	0.451826	0.411582	0.040244
27	0.087907	0.499800	0.455864	0.043936
28	0.219346	0.490950	0.383263	0.107687
29	0.331870	0.499308	0.333603	0.165705
30	0.248709	0.380276	0.285698	0.094578
31	0.125420	0.449013	0.392698	0.056315
32	0.065542	0.497857	0.465227	0.032630
33	0.057099	0.476296	0.449100	0.027196
34	0.087138	0.469938	0.428989	0.040949
35	0.111822	0.497969	0.442285	0.055684
36	0.059603	0.489166	0.460010	0.029156
37	0.197685	0.449607	0.360727	0.088880
All loci	0.130978	0.461184	0.400779	0.060405

Estimates of Shannon's phenotypic diversity (H_o) for RAPD's are given in table 15. They ranged from 0.491 (Uganda 5) to 0.770 (Uganda 9) and the mean for the 15 Uganda populations was 0.619. Estimates of H_o also varied among primers with OPO18 detecting the lowest (0.579) and OPA17 detecting the highest 0.642 value of diversity (table 14). Shannon's information index of phenotypic diversity was used to partition the diversity into within - and between population components (table 15). H_{pop} provided a measure of the average diversity of the fifteen populations. OPO20 detected the most and OPO19 the least within- population variability. A t-test was carried out to evaluate differences between genetic distances within and between *C. sordidus* populations. The statistical result showed that the variation within and between *C. sordidus* populations was significantly different (p<0.0001). An examination of genetic diversity present within populations (H_{pop}/H_{sp}), and between populations, $(H_{sp} -H_{pop})/H_{sp}$, indicated that on the average the diversity (84.7%) mainly occurred within *C. sordidus* populations. The distribution of variability between - and within - populations, however, varied between primers. For example, OPO19 detected most variability between populations (16.6%), whereas primer OPO20 and OPA17 detected most variation within populations (85.2% and 85.1%), respectively.

The genetic diversity within and among the 15 populations revealed average level of divergence (Gst value of 0.131) (table 19). This means that approximately 13% of the genetic variation observed in this study was due to differentiation among populations of *C. sordidus* compared with 87% within populations. Estimates of gene flow (Nm) (table 19) ranged from 1.305 to 1.553. Over all populations, according to Crow and Aoki (1984), gene flow values (Nm) of less than 1 should be interpreted as little or no gene flow. Thus, the average of the values (Nm= 1.45) observed within populations in this study would indicate reasonable migratory events.

Table 14 : Estimates of Shannon's phenotypic diversity index for RAPD's (H_o) for fifteen populations[a] of Banana weevils.

[a]UG2= Kalegweri, UG3= Ikulwe, UG4= Ntengeru, UG5= Buwama, UG6= Bukanga, UG7= Buzaya, UG8= Lakeshores, UG9= Bunyaruguru, UG10= Namubiru, UG11= Ntungamo, UG12= Kabukye, UG13= Bundagara, UG14= Namunonyi, UG15= Kawanda, UG16= Ntinkalu.

PRIMER	UG 2	UG 3	UG 4	UG 5	UG 6	UG 7	UG 8	UG 9	UG 10	UG 11	UG 12	UG 13	UG 14	UG 15	UG 16	MEAN
OPA17	0.751	0.417	0.627	0.359	0.667	0.405	0.496	0.768	0.919	0.578	0.617	0.652	0.717	0.866	0.805	0.642
OPO18	0.770	0.452	0.527	0.404	0.711	0.497	0.452	0.602	0.641	0.736	0.554	0.467	0.749	0.609	0.516	0.579
OPO19	0.469	0.835	0.350	0.325	0.578	0.416	0.821	0.887	0.650	0.502	0.803	0.715	0.544	0.671	0.813	0.624
OPO20	0.544	0.401	0.519	0.895	0.498	0.690	0.379	0.834	0.605	0.721	0.588	0.716	0.529	0.718	0.799	0.629
MEAN	0.633	0.526	0.506	0.491	0.614	0.502	0.537	0.770	0.704	0.634	0.641	0.638	0.635	0.716	0.733	0.619

Table 15: Components of genetic diversity in *C. sordidus* from 15 locations from Uganda, partitioned into within and between populations from 4 random oligonucleotide. H_{pop}=Average diversity of RAPD markers for all populations, H_{sp} = Shannon's within species diversity index, H_{pop}/H_{sp} = within population diversity, $(H_{sp}-H_{pop})/H_{sp}$= diversity among populations.

Primer	H_{pop}	H_{sp}	H_{pop}/H_{sp}	$(H_{sp}-H_{pop})/H_{sp}$
OPA17	0.642	0.754	0.851	0.149
OPO18	0.579	0.684	0.846	0.154
OPO19	0.624	0.748	0.834	0.166
OPO20	0.629	0.738	0.852	0.148
MEAN	0.619	0.731	0.847	0.153

3.3. Molecular markers of randmonly amplified polymorphic DNA (RAPD) for Banana Weevils from different regions of the world.

3.3.1. Polymorphic bands

The amplification results were routinely repeatable. The clusters were distinguished by their RAPD patterns with all primers, however no single primer generated patterns for each cluster. Primer OPO14 produced a unique 500 bp in 2nd cluster designated as 14-1 (figure 8a). Primer OPA17 produced 3 products: F3 approximately 550 bp product designated as 17-1, F2 approximately 500 bp designated as 17-2 and F1 approximately 200 bp designated 17-3 all unique in 1st cluster (figure 8b). Primer OPO18 produced two unique products. A 250 bp product designated as 18-1 unique in 3rd cluster and a 400 bp product designated as 18-2 unique to 6th cluster (figure 8c). Primer OPO19 produced two unique products. A 250 bp product designated as 19-1 unique in 4th cluster and a 600 bp product designated as 19-2 unique in 6th cluster (figure 18d). Primer OPO20 produced a unique 200 bp product designated as 20-1 in 3rd cluster (figure 18e).

3.3.2. Cloning polymorphic bands

The polymorphic PCR products obtained in section 4.5 were ligated with pGEM-T vectors (Promega Corporation, Madison, U.S.A) then transformed into E.coli. PCR screening of the white colonies was done. Two samples were picked from each plate for each of the following fragments 14-1, 17-1, 17-2, 17-3, 18-1, 18-2, 19-1, 19-2, 20-1. Fragment 17-3 did not give any product with pGEM -T primers. All the other fragments were positive (fig.9).

The plasmid DNA was harvested by Wizard SV miniprep kit and checked by running 5µl on 1% agarose. Plasmid DNA in the case of fragment 17-3 was lower than 2 kb, all the rest of the plasmids containing the other fragments were all approximately 2 kb (fig. 10). The plasmid DNA prepared from bacteria harbouring putative recombinant plasmids were digested with EcoRI to confirm presence of inserts, electrophoresed in an

agarose gel, which was subsequently stained with ethidium bromide and photographed (figure 11). Plasmids containing fragment 17-3 on restriction did not give any product. Plasmid containing OPO 19-2 gave two fragments of 400 bp and 200 bp indicating the presence of internal recognition site by EcoRI in the fragment. All the rest of the putative recombinant plasmids had inserts of approximately 500 bp, 400 bp, 500 bp, 250 bp, 400 bp, 400 bp and 200 bp in sizes corresponding to the weights of the original seven fragments 14-1, 17-1, 17-2, 18-1, 18-2, 19-1 and 20-1 respectively that were cloned confirming the presence of the inserts. After restricting the plasmid to release the insert in the case of fragment 17-1 (550 bp), a fragment of 400 bp was observed. This can be attributed to the enzyme having internal restriction site within the fragment giving the 400 bp fragment and additional small fragments that are not resolved on the gel system used.

The polymorphism of the seven fragments 14-1, 17-1, 17-2, 18-1, 18-2, 19-1 and 20-1 was confirmed by Southern blotting of PCR products of the six clusters after separation on agarose gels, and probing with each of the cloned fragments labeled with DIG. The probing revealed the expected approximately 500 bp product for the fragment 14-1 generated by OPO 14 in the 2nd cluster. It did not hybridize to any of the other clusters (figure 12). Fragment 17-1 revealed more products than expected. A product of approximately 550 bp was expected only in 1st cluster but the product was revealed in all clusters except 6th cluster, indicating that this fragment was not very specific for cluster one as indicated by PCR (figure 13). Fragment approximately 500 bp from OPA17 (17 – 2) revealed a product approximately 500 bp in 1st and 4th cluster and did not hybridize to the other clusters (figure 14). Fragment approximately 400 bp from OPO18 revealed a product approximately 400 bp in 6th cluster as expected but did not hybridize to the rest of the clusters (fig.15). Fragment approximately 250 bp produced by OPO19 revealed a product approximately 250 bp as expected in 4th cluster, but did not hybridize to any other cluster (figure 16).

Figure 8:

The results of pooled DNA of individual clusters distinguished by their RAPD patterns with different primers. They were run on 1.2% agarose gels and stained with ethidium bromide after PCR.

A – six clusters amplified by OPO14.

f – 1st cluster, e – 2nd cluster, d – 3rd cluster, c – 4th cluster, b – 5th cluster, a – 6th cluster. F – fragment 14 – 1, M – Molecular weight marker. This order of clusters was maintained for figures 10A – 10E.

B – six clusters amplified by OPA17 (F_1, F_2, F_3 represent fragment 17 – 3, 17 – 2 and 17 – 1 respectively).

C – six clusters amplified by OPO18 (F1 and F2 represent fragment 18 – 1 and 18 – 2 respectively).

D – six clusters amplified by OPO19 (F1 and F2 represent fragment 19 – 1 and 19 – 2 respectively).

E – six clusters amplified by OPO20 (F represents fragment 20 -1).
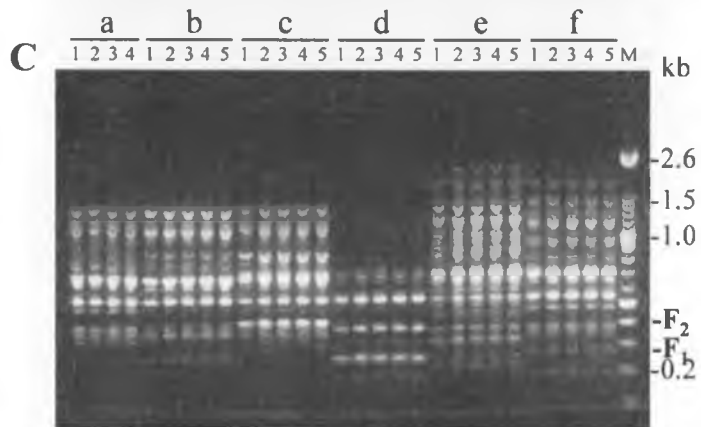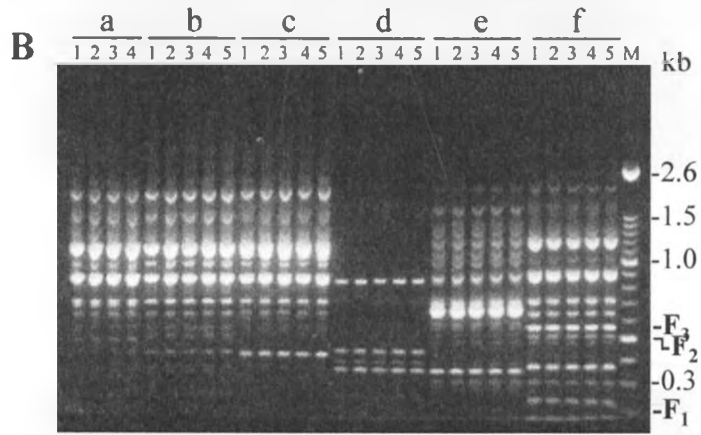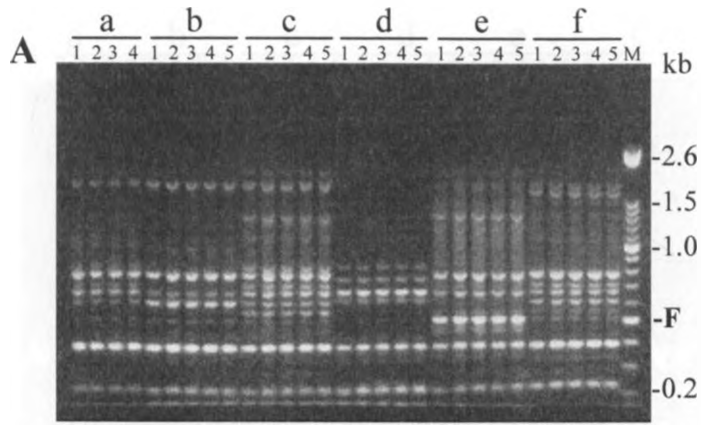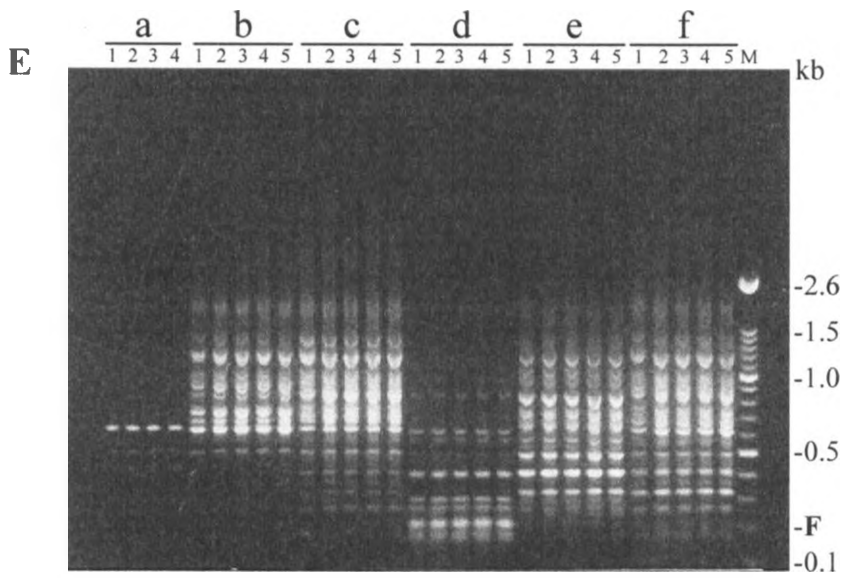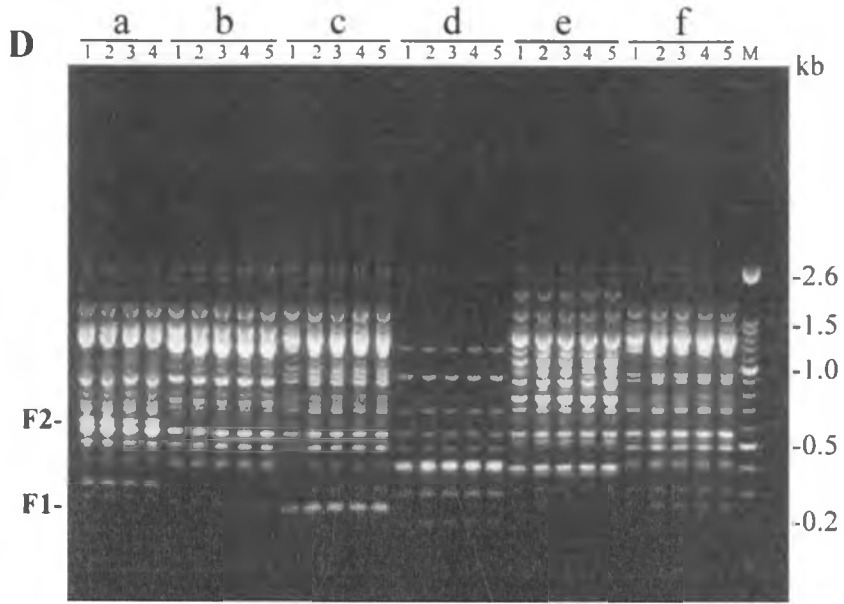
Figure 9:

Results of PCR of the white colonies using PGEM-T primers run on a 1.2% agarose gel and stained with ethidium bromide. Two samples were picked from each plate. 1 – fragment 20 – 1, 2 – fragment 19 – 2, 3 – fragment 19 – 1, 4 – fragment 18 – 2, 5 – fragment 18 – 1, 6 – fragment 1 – 3, 7 – fragment 17 – 2, 8 – fragment 17 – 1, 9 – fragment 14 – 1, M – Molecular weight marker.
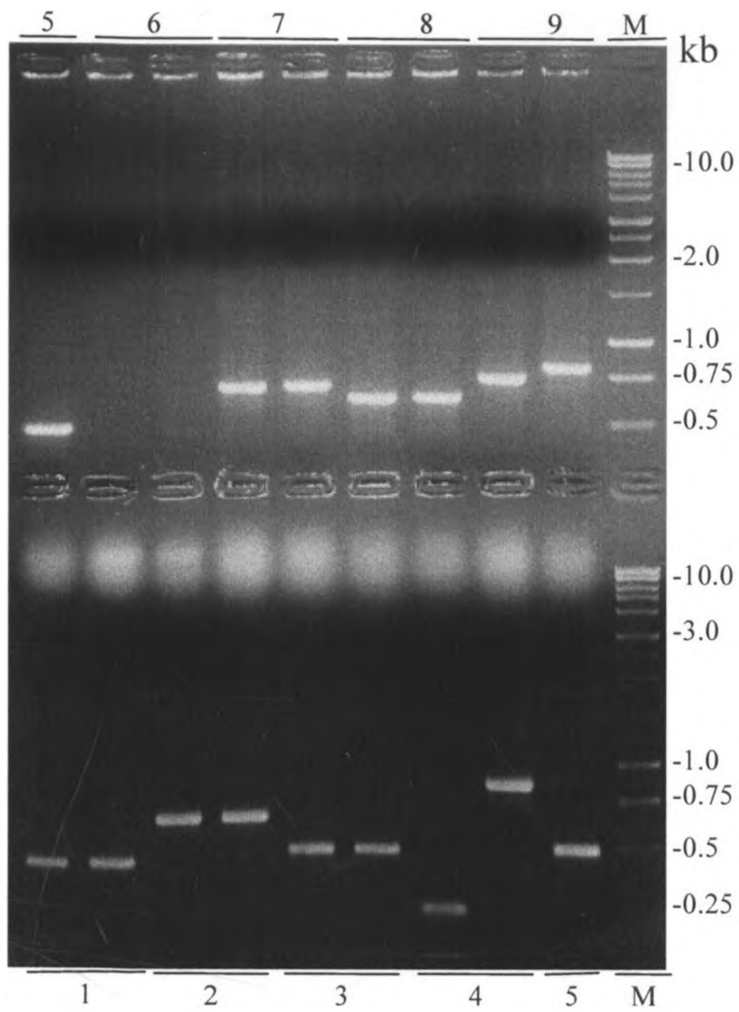
Figure 10:

Plasmid DNA harvested by Wizard S V miniprep kit and checked by running on 1% agarose gel. 1 – fragment 20, 2 – fragment 19 – 2, 3 – fragment 19 – 1, 4 – fragment 18 – 2, 5 – fragment 18 – 1, 6 – fragment 17 – 3, 7 – fragment 17 – 2, 8 – fragment 17 – 1, 9 – fragment 14 – 1 and M – Molecular weight marker.
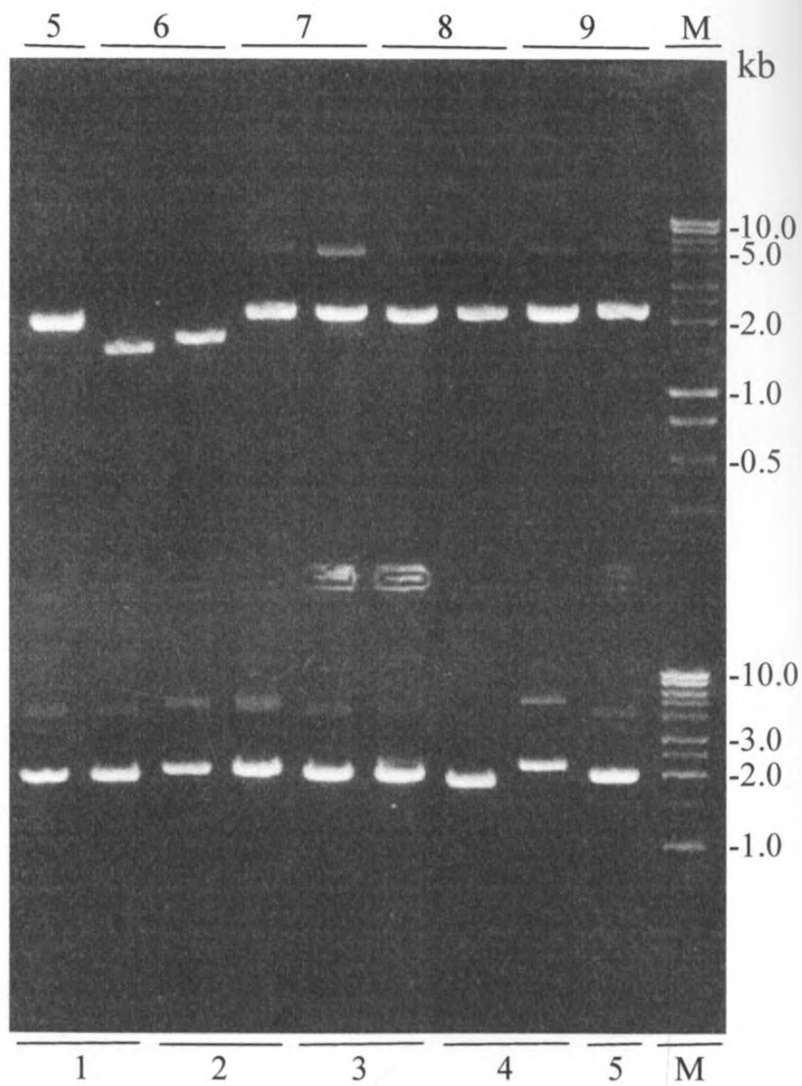
Figure 11:

Showing the confirmation of the presence of inserts after digesting the putative recombinant plasmids with EcoR I run on a 1.2% agarose gel and stained with ethidium bromide.

a – fragment 20 – 1, b – fragment 19 – 2, c – fragment 19 – 1, d – fragment 18 – 2, e – fragment 18 – 1, f – fragment 17 – 3, g – fragment 17 – 2, h – fragment 17 – 1, i – fragment 14 – 1 and M – Molecular weight marker.
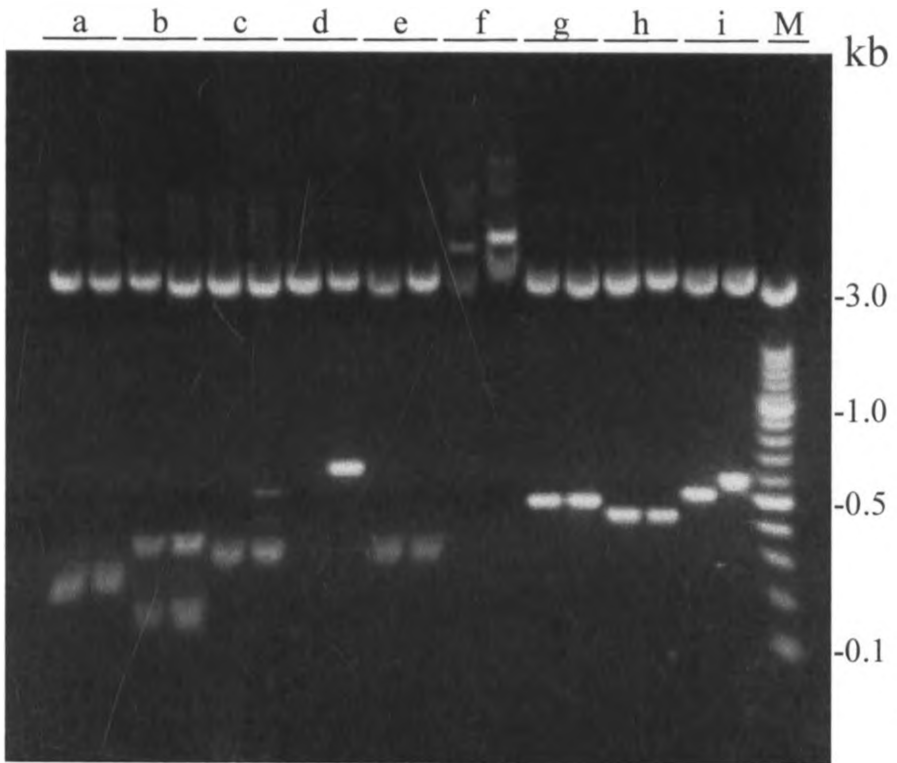
3.3.3. Sequencing and sequence alignment

The seven RAPD products, which were polymorphic were cloned and sent to ILRI for sequencing (figure 17a). A search on the <u>Gene Bank</u> 79 and EMBL 36 databases did not reveal significant homology with any of the accessions. When the fragments were aligned to check for any similarity using Multiple Sequence Alignment with Hierarchical Clustering Program MULTALIN, all the fragments were different (Corpet, 1988) (fig.17b).

Suitable primers were designed for each of the seven fragments and used for PCR studies (table 16).

Figure 12:

Southern blot of PCR products of the six clusters probed with DIG labeled
fragment 14 – 1. 1 and 2 – negative controls, 3 – cluster six, 4 – cluster 5,
5 – cluster 4, 6 – cluster 3, 7 – cluster 2 and 8 – cluster 1.
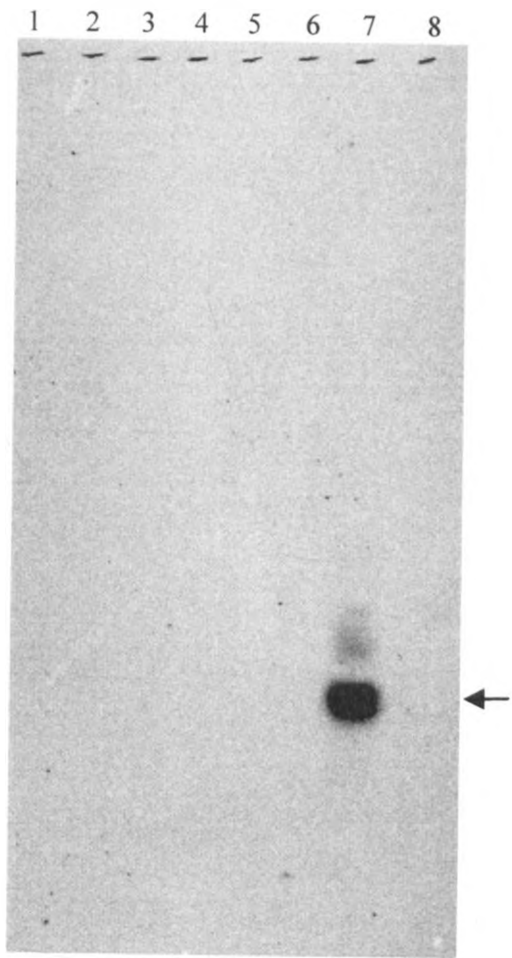
Figure 13:

Southern blot of PCR products of each of the six clusters probed with DIG labeled fragment 17 – 1. 1 – cluster 1, 2 – cluster 2, 3 – cluster 3, 4- cluster 4, 5 – cluster 5 and 6 – cluster 6.
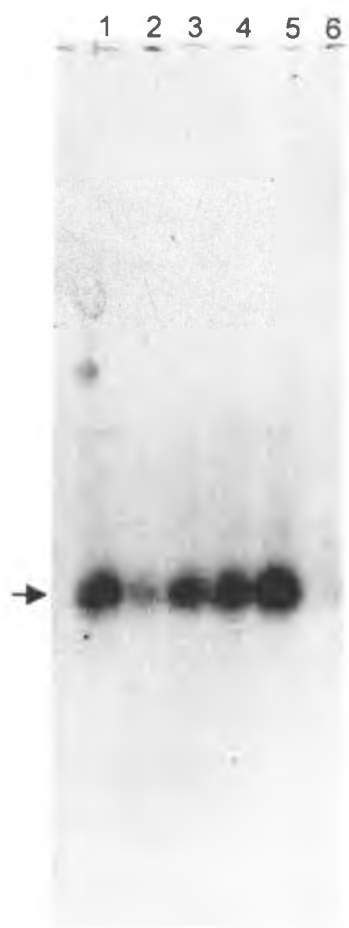
Figure 14:

Southern blot of PCR products of each of the six clusters probed with DIG labeled fragment 17 – 2. 1 – cluster 1, 2- cluster 2, 3 – cluster 3, 4 – cluster 4, 5 – cluster 5 and 6- cluster 6.
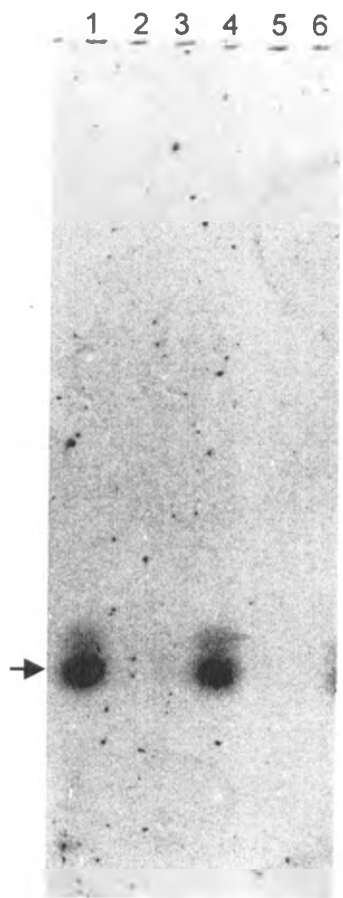
Figure 15:

Southern blot of PCR products of each of the six clusters probed with DIG labeled fragment 18 – 2. 1- cluster 1, 2 – cluster 2, 3 – cluster 3, 4 – cluster 4, 5 – cluster 5, 6 – cluster 6, 7 and 8 - negative controls.
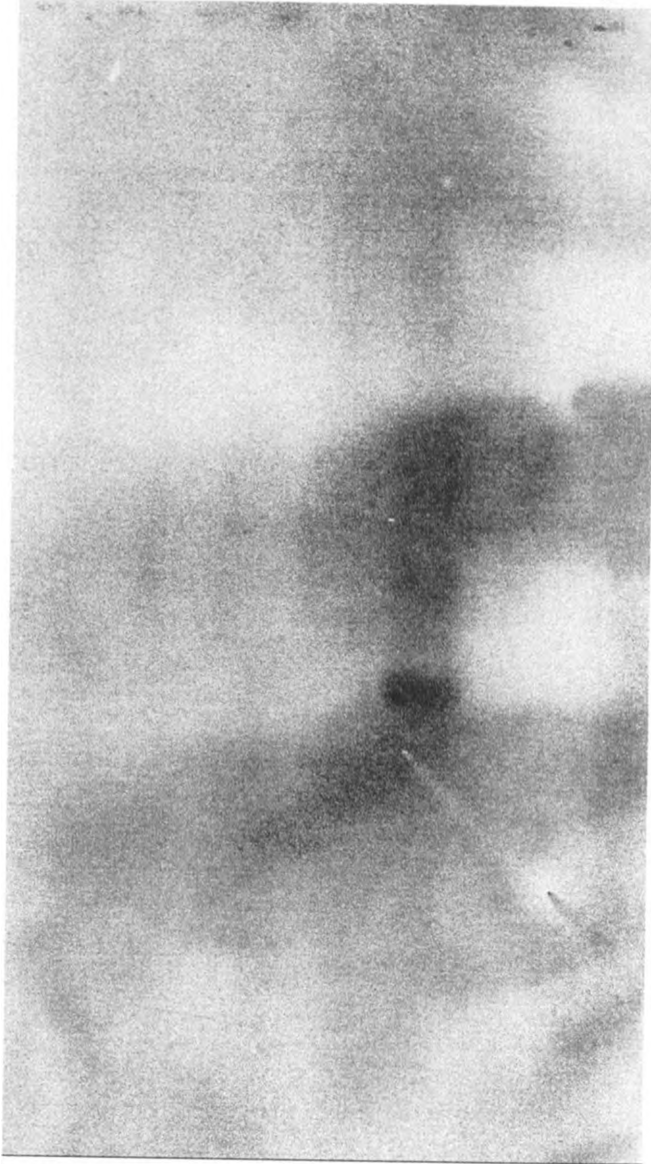
Figure 16:

Southern blot of PCR products of each of the six clusters probed with DIG labeled fragment 19 – 1. 1- cluster 6, 2 – cluster 5, 3 – cluster 4, 4 – cluster 3, 5 – cluster 2, 6 – cluster 1 and B – negative control.
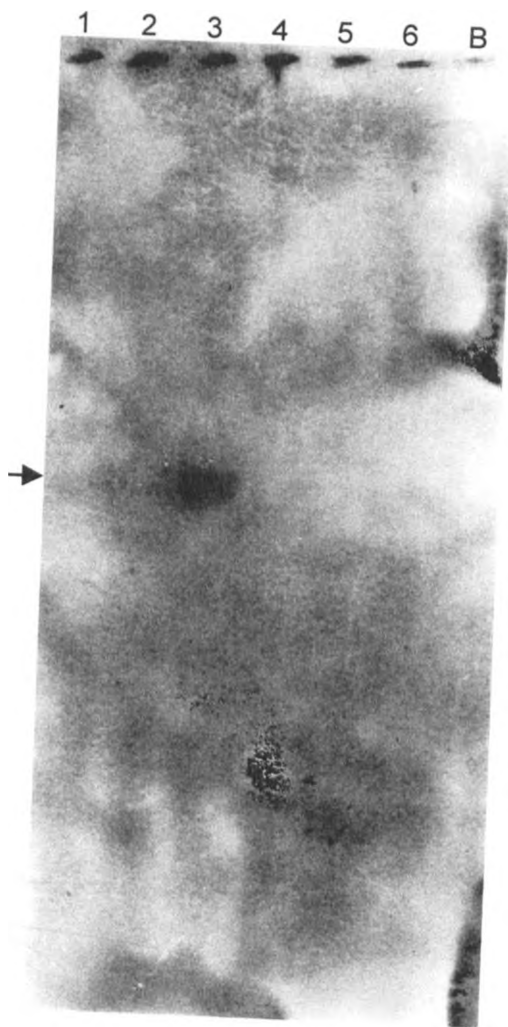
Figure 17a:

Sequences of the seven cloned RAPD fragments. VO-14-1 → fragment 14 –1, VO-17-1 → fragment 17-1, VO-17-2 → fragment 17-2, VO-18-1 → fragment 18-1, VO-18-2 → fragment 18-2, VO-19-1 → fragment 19-1, VO-20-1 → fragment 20-1.

VO-14-1
GATTAGCATGGCTCCGGCCGAGGCAGTAGAATTTCTCAAATTTGGTAACCTCCGTTTTGT
GAATGGCCTGGCCAAACACAAAAATTACCTGGAACGCGTAGAAGAAACCAAGAGCAGCCA
AAAACCATTTGCGGCCATCCTCAGTTGTATGGATAGCCGGGCACCTGTAGAAATCCTGTT
CGACCAGGGCATAGGCGATATATTCAGTGTACGCATGGCGGGAAACATCATTTCCGAAAA
CATTCTGGGATCGCTGGAATATGCCACCGGCGTGGTTGGTTCCAAACTCATCCTGGTAAT
GGGCCACACCAATTGCGGAGCTGTAAAAGGCGCTTGCGATGGAGTGGAATTAGGCCACGT
TACCATCCTGCTGGATAAAATAAAACCGGCAGTGGCGCAGGCCAAAGCGGAAGGCAATGC
CGAGGGTGGTTCAGAATTTTACGAAGAAGTGGCCAGGCTCAATGTGATCAATAGCCTCAA
TGCCATTGTGGAACAAAGCGAAATCATCAGAAACCTGGTGGAAGAAGGTAAAGTACTCCT
GCGTGGAGCCATGCTAATC1

VO-17-1
GATTGACCGCTTGTATGATTAAATGGAAAACCCGTCTATATGTATAGAATTACTAAAGAA
CACCACGTACACTACTGGAGAGATACCTATAAGCAGGGGAATATTTCAGTGGGATAGTTT
AAGATAGGTATGTATGGCTTTAACATCGTTAAGTAGACTGTTGAGAGACTCCAGCATGAG
CTATGCGATGAATAGAAATCCAAACATTCAGGTATCACATCAGTTCTCGATGGACGATCT
AAAATCATATGTATCAAATTCTTATAATCTTCAAAATGAAATAGAGCTTGTCAAAAATGT
TAGCAATACCATCTCCATGGAGTTTGCCCTTGAGAAGTGTGCAGTCGTCCATGTCCACAA
GCGGTCAATC1

V0-17-2
GATTGACCGCTTGTTCAACCACTTTATGATGGTAAATCTGCTCACGAAGTAGTTCAGCTT
TTTGCCAAGGAAAACTTTGAAAAGAAAGAACTTGACATCGTTAAGGATTATTGGAAAGCG
AATGGTTTAGCAGACGATAAATCTTGGAGAAAAGCCGTTCACGATGGTTTTATTCCAAAT
TCGGCATTGCCTGCCAAAGCTGTGACAGCTAGTTCGGCATTTTTGAGTCAACCTGCAACT
GCTCCAAGCGGAAGCGGAAAATATGAAATTGCCATTCTGCCTGATCCGAGTATTTACGAC
GGACGATATTCAAATAACGGCTGGCTCCAAGAAGTTCCAAACCCATTAACCAAAATAACT
TGGGATAACGTGGCATTGGTTTCGCCAAATACTGCCAAAGAACTTGGAGTAAACCAAGGA
NGCGATCCCGAATCACAAGCGGTCAATC1

VO-18-1
GATTCTCGCTATCCACACCGGGCCTTCCGTGAATTTCAAATCGCCCGCCAGCTTTTTGAT
CTCTACATCCGCTTCTCCAATCTTCGCGAAAGCATCGGCATCTTCGATCTTAACGTCCGC
ATGGGCAGAAAACCTCAGCAGAGCACTGTAAAACAGGGCGGAAAAATACTTGATTTTTTC
GTATTGACTTTGCATATACATGGTGTATTGATTGTTCACAGGTTACGGGATAGCGAGAAT
C1

VO-18-2
GATTGGTGCACGTTGCGTATTAAATGCGTAGTAATAGGTTCCTAATTGGTCTGGTGTGAA
AACCTCATTAGGAATATCTTGCAATAACTTTTGATAGCGCTGTTTCGGGAAGGATTCAGT
AATGTCTAAATCCCCAGCTAAATAACGGTTTGTCGCATGAGATTCTTGGTTAATAGGAAT
AAATGTGACTTGAGTAATCACTGTATTTTTATGATCCCAATAATACGGGTTCGGTGTTAA
AACAATTTTTTCATTCACAACACGGTCAGCTAACGTAAAAGCGCCATTTCCAACTAAATT
CCCTACTTTTATCCAATCCGCTCCTAATTTTTCTATCGTTCCACGATGAACTGGATACAA
ACTAAAATTTGCAGCTAAAGAGGGAAAATATGGCACGGGTTTATTCAATGTTATTTTTAA
TGTGTGTTCATCAACCGCTTCCACACCTAATTGCTCCACGGACAACTTGCCATCAATAAT
ATCTTGCGCATTATTAATTCCGGCAAGTGCAGCAAACCATGCAAACGGTGATGTATTTGC
AGGTGCAACCAAACGCTGCCAACTATAAACAAAGTNACTTGCGGTGACAGGCTCTCCATT
GGACCAACGTGCACCAATC1

VO-19-1
GATTGGTGCACGTTCCAAATAATCCAAAATCGCATCAATTTTAGCATTGGTTTTATTGGT
GCTTTCCAAAGCGTTGATGAGTTCTGCGAAATTCTTCATAACTCTGGATTTAAAGTAGTT
TCCTTTTCAGTTACCTCTTCATCATCGTCTCCGTATAAAGTTTCCACGACATCAGATTTA
ATTCCGATTTCGTTCAGATATTTCGAGAAAACTTCGGTTTGTCCGTGCGTAACGTGCACC

AATC1

VO-20-1
GATTACACACGCTGCACAAGAAGGGAGATAGAACAGATGTGAATAACTACAGAGGAATCT
ATTTACTACAAGTTACTTACAAAATCCTCTCAGCATGCCTTCTTCAGAGAACACAAGAAC
AATTAGAACACAAAATTGGCGAGTAACCTTTCGTTCAAGTTAAGTTGTGAAGTTTTCCTG
GTTGGATCAGCGTGTGTAATC1

Figure 17b:

Sequenced alignment of the 7 polymorphic fragments using MULTALIN.

```
          1       10        20        30        40        50        60        70        80        90       100       110       120      130
          |-------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
          |                                                                                                                              |
VO-14-1   GATTAGCATGGCTCCGGCCGAG-GCAGTAGAATTTCTCAAATTTG------GTAACCTCCGTTT-TGTGAATGGCCTGGCCAAACACAAAAATTACCTGGAACGCGTAGAA-------GAAACCAAG
VO-18-2   GATTGGTGCACGTTGCGTATTAAATGCGTAGTAATAGGTTCCTAATTGGTCTGGTGTGAAAACCTCAT-TAGGAATATCTTGCAATAACTTTTGATAGCGCTGTTTCGGGAAGGATTCAGTAATGTCTAA
VO-17-2                                                                                                                             GATTG
VO-17-1                        GATTGACCGCTTGTATGATTAAATGGAAAACCCGTCTATATGTATAGAATTACTAAAGAAC----ACCACGTAC
VO-19-1
VO-18-1
VO-20-1
Consensus .............................................................................................................................

          131     140       150       160       170       180       190       200       210       220       230       240       250      260
          |-------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
VO-14-1   AGC---AGCCAAA-AACCATTTGCGGCC----ATCCTCAGTTGTATGGATAGCCGGGCACCTGTAGAAATCCTGT----------TC------------GACCAGGGCATAGGCGATATATTCAGTGTAC
VO-18-2   ATCCCCAGCTAAATAACGGTTTGTCGCATGAGATTCTTGGTTAATAGGAATAAATGTGACTTGAGTAATCACTGTATTTTTATGATCCCAATAATACGGGTTCGGTGTTAAAACATTTTTTTCATTCACA
VO-17-2   ACCGCTTGTTCAACCACTTTATGATGGTAAATCTGCTCACGAGGTAGTTCAGCTTTTTGCCAAGGAAAACTTTGAA---------------AAGAAAGAACTTGACATCGTTAAGGATTATTGGAAAGC
VO-17-1   ACTACTGGAGAGATACCTATAAGCAGGGGAATATTTCAGTGGGATAGTTTAA--GATAGGTATGTATGGCTTT----------------------------AACATCGTTAAGTAGACTGTTGAGAGACTCC
VO-19-1
VO-18-1                                                                                                           GATTCTCGCTATCCACACCGGGCCTTCCGT--
VO-20-1                                                                                                           GATTACACACGCTGCACAAGAAGG--
Consensus a.......g....a...c..t..g.g........t.............g.............................t...............g..ta...aca.t.t...........

          261     270       280       290       300       310       320       330       340       350       360       370       380      390
          |-------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
VO-14-1   -GCATGG-C-GGGAAACATCATTTC-CGAAAACATTCTGGGATCGCTGGAATATGCCAC-CGGCGTGGTTGGTTCCAAACTCATCCTGGT-AATGGGCCACACCAATTGCGGAGCTGTAAAAGGCGC-TT
VO-18-2   -ACACGGTC-HGCTAACGTAAAGCGCCATTTCCAACTAAAATCCCTACTTTTTATCCAATCCGCTCCTAATTTTTCTATCGTTCCACGATGAACTGGATACAAACTAAAATTTGCAGCTAAAGAGGG-AA
VO-17-2   -GAATGGTTTAGCAGACGATAAATCTTGGAGAAAAGCCGTTCACGATGGTTTTATTC---CAAATTCGGCATTGCCTGCCAAAGCTGTGACAGCTAGTTCGGCATTTTTGAGTCAACCTGCAACTGC-TC
VO-17-1   AGCATGAGCTATGCGATGAATAGAATCCAAACATTCAGGTATCACATCAGTTCT--------CGATGGACGATCTAAAATCATATGTATCAAATTCTTATAATCTTCAAAATGAAATAGAGCTTGT-CA
VO-19-1            GATTGGTGCACGTTCCAAATAATCCAAAATCGCATCAATTTTTAGCATTGGTTTTATTGGTGCTTTCCAAAGCGTTGATGAGTTCTGCGAAATTCTTCATAACTCTGGATTTAAAGTA
VO-18-1   -GAATTTCAAATCGCCCGCCAGCTCTTTTTGATCTCTACATCCGCTTCTCCAATCTTCGCGAAAGCATCGGCATCTTCGATCTTAACGTCCGCATGGGCAGAAAACCTCAGCCGGAGCACTGTAAAACAGGGC
VO-20-1   -GAGATAGAA---------CHGATGTGA-ATAACTACAGAGGAATCTATTTACTACAAGTTACTTACAAAATCCTC----------TCAGCATG--------CCTTCTTCAGAGAACACAAGAACAATTA
Consensus .g.atg....a....acg..a.at.tt..a.a.at.t.g...tc.ct.ca.ttttc......gcttcg...tttcc.a.c..a.c.t...caa.t....a.aa..t.t.cagagcact..aa...g..ta

          391     400       410       420       430       440       450       460       470       480       490       500       510      520
          |-------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
VO-14-1   GCGATGGGAGTGGAATTAGGCCACGTTACCATCCTGCTGGATAA-HATAAAACCGGCAGTGGCGCAGGCCAAAGCGGAAGGCAATGCCGAGGGTGGTT--------CAGAATTTTTACGAAGAAGTGGCCAGG
VO-18-2   AATATGGCACGGGTTTATTCAATGTTATTTTTAATGTGTGTTC-ATCAACCGCTTCCACACCTAATTGCTCCACGGACAACT-TGCCATCAATAATATCTTGCGCATTATTAATTCCGGCAAGTGCAGCA
VO-17-2   CAAGCGGAAGCGGAAAATATGAAATTGCCATTCTGCCTGATCCGAGTATTTACGACGGACGATATTCAAATAACGGCTGGCT--CCAAGAA-----------------GTTCCAAACC-----CATTA
VO-17-1   AAAATGTTAGCAATACCATCTCCATGGAGTTTGCCCTTGAGAAGTGTGCAGTCGTC-----CATGTCCACAAGCGGTCAATC
VO-19-1   GTTTCCTTTTCAGTTACCTCTTCATCATCGTCTCCGTATAAAGTTTCCACGACATCAGATTTAATTCCGATTTCGTTCAGATATTTCGAGAAAA----------CTTCGGTTTGTCCGTGCGTAACGTGC
VO-18-1   GGAAAAATACTTGATTTTTTCGTATTGACTTTGCATATACATGGTGTATTGATTGT--TCACAGGTTACGGGATAGCGAGAATC
VO-20-1   GAACACAAAATTGG------CGAGTAACCTTT-CGTTCAAGTTAAGTTGTGAAGTT--TTCCTGGTT--GGATCAGCGTGTGTAATC
Consensus gaaa.g...a...g.t...tc...aTta.ctTt.c..t..a....agta..gacgtc.....c...t.....a.cgg...ag.......c..............................c....

          521     530       540       550       560       570       580       590       600       610       620      627
          |-------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+------|
VO-14-1   CTCAATGTGATCAATAGCCTCAATGCCATTGTGGAACAAAGCGAAATCATC-AGAAACCTGGTGGAAGAAGGTAAAGTACTCCTGCGTGGAGCCATG----CTAATC
VO-18-2   AACCATGCAAACGGTGATGTATTTGCAG--GTGCAACCAAACGCTGCCAACTATAAACAAAGTNACTTGCGGTGACAGGCTCTCCATTGGACCAACGTGCACCAATC
VO-17-2   ACCAAAATAACTTGGGATAACGTGGCATTGGTTTCGCCAAATACTGCCA---AAGAACTTGGAGTAAACCAAGGANGCGATCCCGAATC-ACAAGCG---GTCAATC
VO-17-1
VO-19-1   ACCAATC
VO-18-1
VO-20-1
Consensus ...u.a..............................................................................................
```

TABLE 16. BASE-PAIR SEQUENCE DESIGNED PRIMERS

PRIMER	BASE-PAIR SEQUENCE (5'-3') Top sequence – forward and bottom – reverse
VO 14.1	GGC CGA GGC AGT AGA ATT TCT C ATG GCT CCA CGC AGG AGT AC
VO 17.1	GAC CGC TTG TAT GAT TAA ATG G GGA CAT GGA CGA CTG CAC AC
VO 17.2	GTT CAA CCA CTT TAT GAT GG GCT TGT TCG GGA TCG C
VO 18-1	CCA CAC CGG GCC TTC CGT G CCG TAA CCT GTG AAC AAT C
VO 18-2	GTG CAC GTT GCG TAT TAA ATG C GTT GGT CCA ATG GAG AGC CTG
VO 19-1	CCA AAT AAT CCA AAA TCG CAT C CGC ACG GAC AAA CCG AAG
VO 20-1	GCA CAA GAA GGG AGA TAG AAC CAA CCA GGA AAA CTT CAC AAC

3.3.4. Polymerase chain reaction (PCR)

In order to obtain the predicted PCR product for each of the fragments 14-1, 17-1, 17-2, 18-1, 18-2, 19-1, 20-1 within each cluster when the primers designed above were used to probe the pooled DNA for each of the six clusters, it was necessary to optimize the PCR amplification conditions. Annealing temperature of 53°C in combination with other conditions of PCR amplification, as described in Materials and Methods section 4.6.7 were used in further experiments involving PCR amplification.

The conditions thus established were used to obtain the results shown in figure 20a, b. Primer VO 14-1 amplified predominantly one major band only in DNA from 2nd cluster giving a product approximately 500bp but did not with the other clusters (figure 20a). Primer VO 18-2 also amplified predominantly one major band only DNA from 6th cluster giving a product approximately 550bp but did not amplify all the other clusters (fig. 20b). It is apparent that no single distinct products were obtained with some primer pairs due to primer annealing at several sites in the genome, thus producing several fragments of different sizes (VO 18-1 and VO 20-1) (fig. 21). Primer OPO19-1 amplified one major product in 4th cluster. VO 17-1 amplified DNA in first, second, third, fourth and fifth clusters and did not amplify the sixth cluster. VO 17-2 amplified DNA from first and fourth clusters only.

3.4. Genetic Population structure of Banana weevils

3.4.1. Genetic population structure of Banana Weevils from different parts of the world.

The genetic distances of Nei (1972) were calculated for all possible pairwise comparisons among 15 populations. These were clustered by UPGMA . Six clusters were generated: Columbia, Zanzibar, Mbita, Tanzania formed the first cluster; Puerto Rico was in the second cluster; Indonesia, Australia, Florida formed the third cluster; Ghana, Embu, Uganda formed the third cluster; Cameroon was in the fifth cluster and the sixth cluster included Honduras, Costa Rica and South Africa (fig.18).

All the banana weevil populations showed distinct RAPD electrophoretic types using the five primers. The four individual (Tanzania, Mbita, Zanzibar, Columbia) were

grouped together at 93% similar level. However, two of these, Tanzania and Mbita, were 96% similar. Puerto Rico was 90% similar to (Tanzania, Mbita, Zanzibar and Columbia). Florida, Australia and Indonesia clustered together at 91% similarity level while two of these Florida and Australia were 93% similar. Uganda, Embu and Ghana grouped together at 89% similarity level and two of these Embu and Ghana were 91% similar. South Africa, Costa Rica, Honduras grouped together at 90% similarity level and two of these, South Africa and Costa Rica were 91% similar. Cameroon was 85% similar to South Africa, Costa Rica and Honduras (fig.18).

Figure 18:

UPGMA cluster analysis of Nei's genetic distance for 15 *Cosmopolites sordidus* populations.

0.2274	0.1895	0.1516	0.1137	0.0758	0.0379	0.000
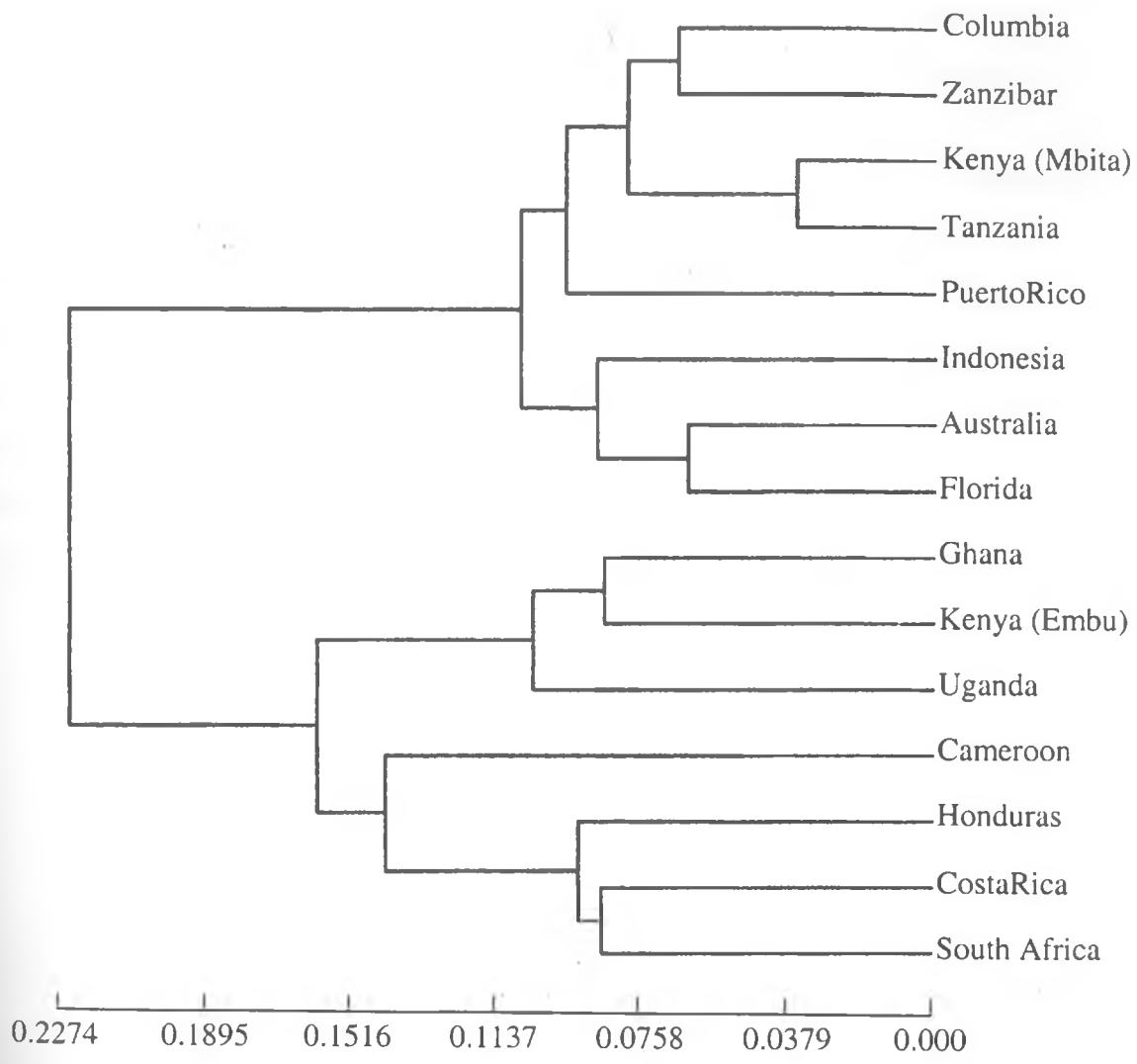
3.4.2. Genetic population structure of Banana Weevils from Uganda.

Samples from different regions of Uganda (fig. 4) were analyzed using four primers OPA 17, OPO 18, OPO 19 and OPO 20. The genetic distances of Nei (1972) were calculated for all possible pairwise comparisons among 15 populations. These were clustered by UPGMA. Six clusters were observed. Kalegweri, Namubiru, Ntungamo, Bukanga, Kabukye and Bundagara formed first cluster. Namunyonyi, Kawanda, Ntinkalu formed the second cluster. Ikulwe and Lakeshores formed the third cluster. Ntengeru was in fourth cluster. Bunyaruguru was in fifth cluster and the sixth had Buwama and Buzaya (fig. 19). A Total of 37 loci were analyzed. Three individuals from Ntungamo, Namubiru and Kalegweri grouped at 95% similarity level, two of these Namubiru and Kalegweri were 97% overall. Bukanga group together with Ntungamo, Namubiru and Kalegweri at 93% similarity level. Bundagara and Kabukye grouped together at 96% similarity level. Namunyonyi, Kawanda and Ntinkalu grouped together at 92%, while Ntinkalu is 96% similar to Kawanda. Ikulwe and Lakeshores were 99% similar. Buzaya and Buwama were 91% similar. All the weevils from all the sites from Uganda were similar at 88% level (fig. 19).

Figure 19:

UPGMA cluster analysis of Nei's genetic distance in Ugandan populations.

							Kalegweri
							Namubiru
							Ntungamo
							Bukanga
							Kabukye
							Bundagara
							Namunyon
							Kawanda
							Ntinkalu
							Ikulwe
							Lakeshores
							Ntengeru
							Bunyarugui
							Buwama
							Buzaya

| 0.1392 | 0.1160 | 0.0928 | 0.0696 | 0.0464 | 0.0232 | 0 |
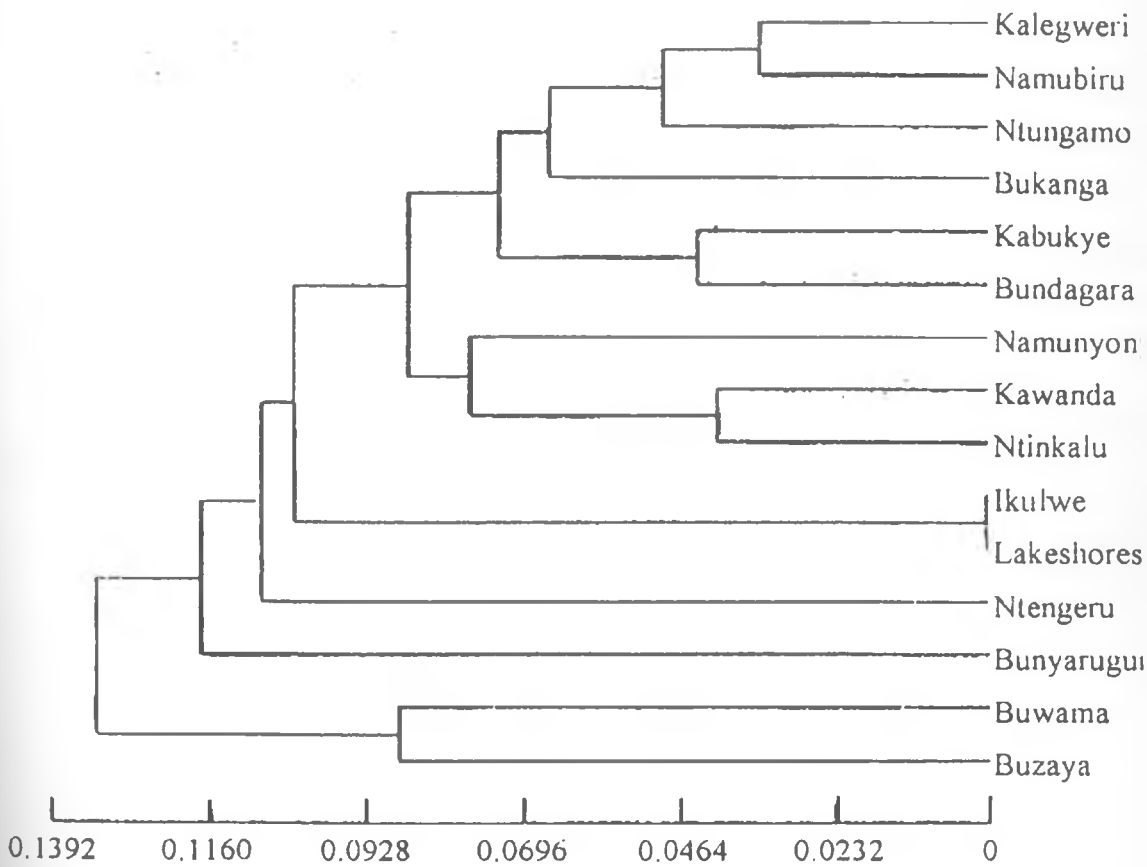
3.5.   Taxonomy and phylogenetic relationships of Banana weevils based on information from -intra-specific differentiation.

3.5.1.   Taxonomy and phylogenetic relationships of Banana weevils from different parts of the world based on intra-specific differentiation.

The coefficient of gene differentiation (Gst) was 0.220 and between population gene diversity (Dst) was 0.102, therefore within population diversity (Hs) was responsible for most of the genetic diversity. The combination of different loci to this partitition of variability indicated that locus 37 and locus 36 contributed the most, and locus 40, 29, 41 and 39 contributed the least to the partitition (table 18b). All the 46 loci were polymorphic and Gst higher than 0.2 were (25 out of the 46) polymorphic loci. Two loci (37 and 36) showed a Gst higher than 0.35 (table 18b).

The smallest genetic distance values were observed between Tanzania + Mbita, Zanzibar + Mbita and Tanzania + Columbia while the distance values between Indonesia and Ghana, Indonesia and Honduras, Kenya (Embu) and Florida, Kenya (Embu) and Australia were much higher.   The highest genetic distance values were observed between Kenya (Embu) and Indonesia (table 18).

Table 17: Nei's genetic diversity values and estimates of gene flow between *C. sordidus* from world populations : Partitioning of total diversity (Ht), for each polymorphic locus and averaged for the 37 loci: diversity within population (Hs), between populations gene diversity (Dst), and proportion of total allelic diversity found among populations (Gst), Gene flow estimates (Nm).

Primer	Dst	Hs	Ht	Gst	Nm
OPO14	0.097	0.357	0.454	0.214	0.8
OPA17	0.099	0.383	0.482	0.206	0.840
OPO18	0.108	0.371	0.478	0.225	0.750
OPO19	0.121	0.345	0.467	0.261	0.617
OPO20	0.082	0.393	0.474	0.172	1.05
Mean	0.102	0.369	0.471	0.216	0.811

Table 18: Similarity matrix computed using Nei's distance for world populations.

matrix: Standard genetic distances

	1	2	3	4	5	6	7	8	9
2	0.2021								
3	0.1346	0.1651							
4	0.2026	0.1142	0.1520						
5	0.0676	0.2160	0.1518	0.2305					
6	0.3307	0.1904	0.2316	0.1413	0.3533				
7	0.2790	0.2104	0.1929	0.1044	0.2945	0.1018			
8	0.0977	0.3004	0.1092	0.3075	0.0882	0.5110	0.4718		
9	0.0943	0.1537	0.0786	0.1363	0.1027	0.1732	0.2049	0.1237	
10	0.3924	0.2777	0.2725	0.1828	0.4011	0.0910	0.1648	0.5525	0.1937
11	0.1199	0.2320	0.0941	0.2257	0.0920	0.3291	0.3431	0.1006	0.0920
12	0.1395	0.1287	0.1359	0.0920	0.1539	0.1425	0.0921	0.2497	0.1156
13	0.1107	0.1642	0.1006	0.1196	0.1190	0.1335	0.1613	0.1831	0.0379
14	0.2387	0.2075	0.1883	0.1731	0.2535	0.0919	0.1053	0.3856	0.1219
15	0.0892	0.1899	0.0706	0.1619	0.1003	0.2501	0.2286	0.0751	0.0669

matrix: Standard error of standard genetic distances

	1	2	3	4	5	6	7	8	9
2	0.0453								
3	0.0369	0.0362							
4	0.0501	0.0260	0.0322						
5	0.0191	0.0467	0.0325	0.0443					
6	0.0550	0.0423	0.0346	0.0261	0.0485				
7	0.0488	0.0474	0.0326	0.0220	0.0467	0.0249			
8	0.0220	0.0565	0.0241	0.0435	0.0170	0.0542	0.0547		
9	0.0221	0.0365	0.0215	0.0272	0.0159	0.0305	0.0325	0.0232	
10	0.0686	0.0714	0.0511	0.0522	0.0559	0.0348	0.0515	0.0769	0.0368
11	0.0301	0.0430	0.0215	0.0462	0.0260	0.0471	0.0627	0.0202	0.0194
12	0.0350	0.0298	0.0212	0.0290	0.0315	0.0242	0.0194	0.0363	0.0277
13	0.0278	0.0371	0.0150	0.0264	0.0217	0.0251	0.0340	0.0289	0.0091
14	0.0466	0.0577	0.0325	0.0373	0.0402	0.0215	0.0293	0.0545	0.0227
15	0.0206	0.0398	0.0155	0.0306	0.0217	0.0331	0.0409	0.0153	0.0193

	10	11	12	13	14

0.3605				
0.2376	0.1994			
0.1790	0.1273	0.1040		
0.1289	0.2619	0.1126	0.1259	
0.3209	0.0908	0.1155	0.0915	0.1909

	10	11	12	13	14

0.0503				
0.0533	0.0389			
0.0506	0.0233	0.0195		
0.0331	0.0476	0.0235	0.0282	
0.0558	0.0263	0.0260	0.0181	0.0327

3.5.2. Taxonomy and phylogenetic relationships of Banana weevils from differer parts of Uganda based on information from intra-specific differentiation.

The coefficient of gene differentiation (Gst) was 0.130. The contribution of different loci to the observed partitition of variability indicated that locus 29 and 30 contributed the most and loci 25, 33, 36 and 15 contributed the least to the partitition (table 18b). A few loci had a Gst higher than 0.2 (5 out of the 37 polymorphic loci). None had a Gst higher than 0.35 (table 13b).

The genetic distance coefficient were averaged among the populations (table 2 The smallest genetic distance values were observed between Ikulwe and Lakeshores, Kalegweri and Namubiru and Ntinkalu and Kawanda. While the distance values betw Buwama and Ikulwe, Lakeshores and Buwama, Bunyaruguru and Ntengeru were muc higher. The highest genetic distance values were observed between Bunyaruguru and Buwama.

Table 19: Nei's genetic diversity values and estimates of gene flow between *C. sordidus* from Uganda populations : Partitioning of total diversity (Ht), for each polymorphic locus and averaged for the 37 loci: diversity within population (Hs), between populations gene diversity (Dst), and proportion of total allelic diversity found among populations (Gst), Gene flow estimate (Nm).

Primer	Dst	Hs	Ht	Gst	Nm
OPA17	0.058	0.390	0.448	0.128	1.483
OPO18	0.0559	0.403	0.454	0.123	1.553
OPO19	0.062	0.412	0.474	0.130	1.457
OPO20	0.066	0.402	0.468	0.143	1.305
MEAN	0.060	0.401	0.461	0.131	1.450

Table 20: Similarity matrix computed using Nei's distance for Ugandan samples.

matrix: Standard genetic distances

	1	2	3	4	5	6	7	8	9
2	0.1022								
3	0.0737	0.1297							
4	0.1269	0.1732	0.1992						
5	0.0522	0.0829	0.1009	0.1441					
6	0.1011	0.1000	0.1165	0.0869	0.1035				
7	0.0933	-0.0080	0.1146	0.1740	0.0667	0.0968			
8	0.1412	0.1276	0.1762	0.1779	0.1184	0.1562	0.1225		
9	0.0341	0.1096	0.1156	0.1000	0.0681	0.0954	0.1010	0.0798	
10	0.0438	0.0940	0.1135	0.1281	0.0749	0.0679	0.0967	0.1444	0.0527
11	0.0577	0.0781	0.0926	0.1560	0.0955	0.1123	0.0715	0.1085	0.0495
12	0.0589	0.0881	0.0582	0.1134	0.0759	0.0771	0.0850	0.0921	0.0512
13	0.0603	0.1192	0.1114	0.1889	0.1027	0.1103	0.1075	0.0984	0.0679
14	0.0632	0.1407	0.0996	0.1536	0.0913	0.1456	0.1300	0.0945	0.0462
15	0.0940	0.1399	0.1675	0.1477	0.1198	0.1469	0.1355	0.0874	0.0765

matrix: Standard error of standard genetic distances

	1	2	3	4	5	6	7	8	9
2	0.0195								
3	0.0194	0.0258							
4	0.0243	0.0469	0.0417						
5	0.0168	0.0193	0.0216	0.0346					
6	0.0263	0.0334	0.0338	0.0216	0.0259				
7	0.0194	0.0020	0.0233	0.0459	0.0158	0.0324			
8	0.0487	0.0309	0.0499	0.0380	0.0368	0.0327	0.0307		
9	0.0102	0.0413	0.0224	0.0202	0.0280	0.0273	0.0405	0.0233	
10	0.0159	0.0289	0.0331	0.0249	0.0230	0.0191	0.0286	0.0370	0.0131
11	0.0146	0.0182	0.0264	0.0296	0.0181	0.0257	0.0179	0.0462	0.0149
12	0.0163	0.0288	0.0123	0.0284	0.0222	0.0202	0.0290	0.0344	0.0120
13	0.0217	0.0243	0.0311	0.0461	0.0374	0.0272	0.0218	0.0294	0.0271
14	0.0147	0.0372	0.0231	0.0430	0.0281	0.0472	0.0329	0.0293	0.0169
15	0.0204	0.0320	0.0353	0.0328	0.0271	0.0283	0.0300	0.0259	0.0196

	10	11	12	13	14

0.1161				
0.0736	0.0431			
0.0938	0.0648	0.0974		
0.1126	0.0658	0.0729	0.0718	
0.1284	0.0894	0.0929	0.0814	0.0402

	10	11	12	13	14

0.0235				
0.0152	0.0114			
0.0316	0.0143	0.0279		
0.0341	0.0210	0.0257	0.0255	
0.0237	0.0232	0.0257	0.0206	0.0132

3.6. PCR-RFLP analysis of the CO1 region of the mitochondria

3.6.1. Restriction Endonuclease Digests

Amplification of the CO1 mitochondrial region using primers S1460 and A2442 produced an amplicon estimated to be 1000 bp (fig. 22a). Primer pair S2442 and A2771 produced an amplicon estimated to be 400 bp (fig. 22a). The amplicons were readily amplified in all the samples used except samples from Indonesia. The CO1 mitochondrial region produced an amplicon estimated to be 1000 bp for all the populations except Indonesia using primers S1460 and A2442 (fig. 22b). The CO1 mitochondrial region resulted in an amplicon estimated to be 400 bp for all the samples from different regions of the world except Indonesia with the primers S2442 and A2771 (fig. 22c).

Fifty-one restriction enzymes were screened (fig. 23 a,b). The restriction enzymes Ava II, Bst E II, Dra III, Hae III, Dde I, Hinf I, Hind II, Hpa II, Nde II, RSA I, Sau I and Xba I restricted the 1000 bp amplicon (fig. 23c). These enzymes were therefore used to examine the genetic differentiation among the different banana weevil populations from different regions. The rest of the enzymes did not cut the amplicon. The 400 bp amplicon was cut using Alu I.

Figure 20:

Shows PCR products obtained using synthesized primers for each fragment run on a 1.2% agarose gel and stained with ethidium bromide. A – primer VO-14-1 was used to analyze DNA from individual clusters. 1 – negative control, 2 – cluster 1, 3 - cluster 2, 4 – cluster 3, 5 – cluster 4, 6 – cluster 5, 7 – cluster 6, M – Molecular weight marker.

B – primer VO-18-2 was used to analyze DNA from individual clusters. 1 and 8 were negative controls, 2- cluster 1, 3 – cluster 2, 4 – cluster 3, 5 – cluster 4, 6- cluster 5, 7 – cluster 6.
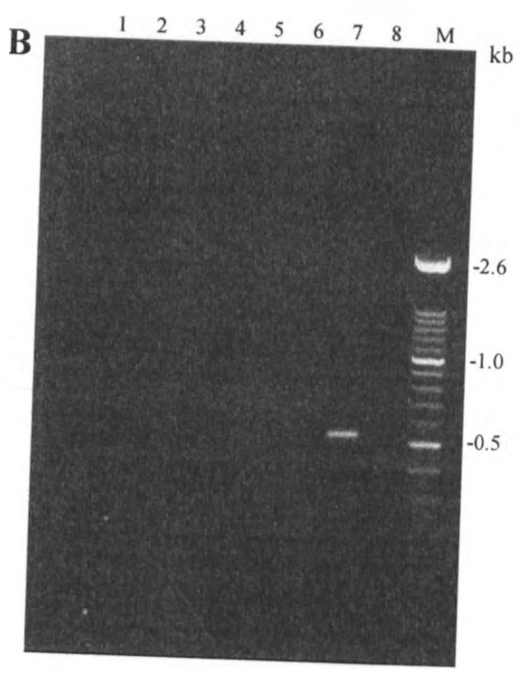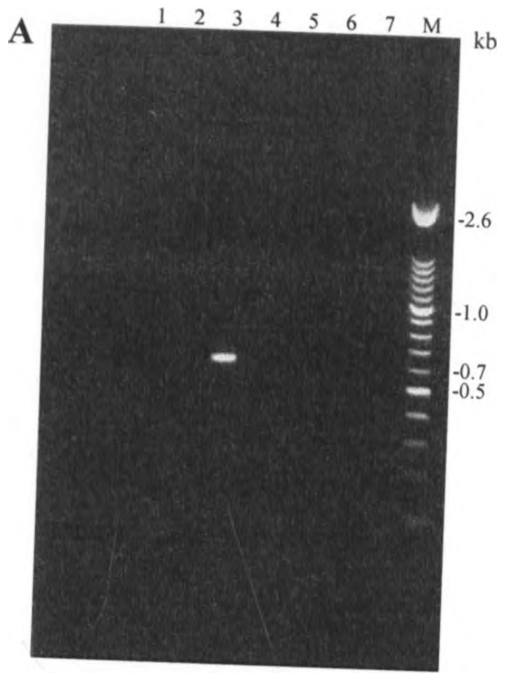
Figure 21:

PCR products obtained using synthesized primers run on a 1.2% agarose gel and stained with ethidium bromide.

A – were products obtained using primer VO- 20-1.

A was done at a lower annealing temperature. 1-cluster 6, 2- cluster 5, 3 – cluster 4, 4-cluster 3, 5-cluster 2, 6- cluster 1, B – negative control.

B – were products obtained using primer VO-18-1, 1-cluster 1, 2-cluster2, 3-cluster 3, 4-cluster4, 5-cluster 5, 6-negative control, 7-cluster 6.

C – were products obtained using primer VO – 20-1 but at higher annealing temperature. 1-cluster 1, 2-cluster 2, 3-cluster 3, 4-cluster 4, 5-cluster 5, 6 –cluster 6, B- negative control, M-molecular weight marker.
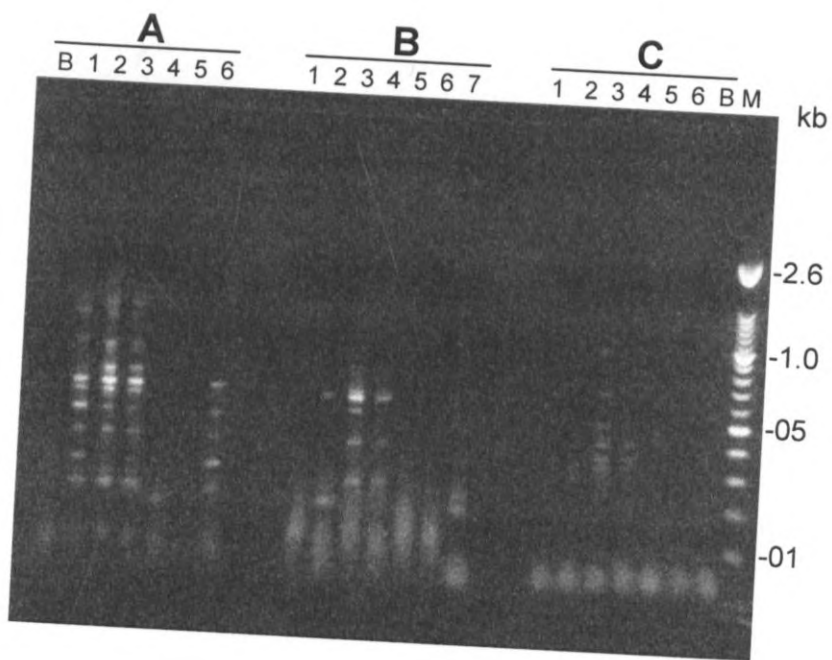
Figure 22:

A- 1.5 % agarose gel showing amplicons obtained by amplifying CO1 mitochondrial region using two pairs of primers. a- S2442 and A2771, b- S1460 and A2442.

B- 1.5 % agarose gel showing amplicons obtained by using primers S1460 and A2442 for samples 1-17 in the order indicated in section 4.8.1.

C- 1.5 % agarose gel showing amplicons obtained by using primers S2442 and A2771 for samples 1-17 in the order indicated in section 4.8.1.

Figure 23:

A and B- 1.5 % agarose gel showing 51 restriction enzymes screened using 1000 bp amplicon. Enzymes 1-27 in A and continuation 1-27 in B are in the same order as given in section 4.8.4. Wells 28 in A and 26 in B are undigested 1000 bp amplicon. Last two wells in both gels are molecular weight markers.

C- 1.5 % agarose gel showing the twelf enzymes that restricted the 1000 bp amplicon. 1-12 are in the same order as given in section 3.8.1.
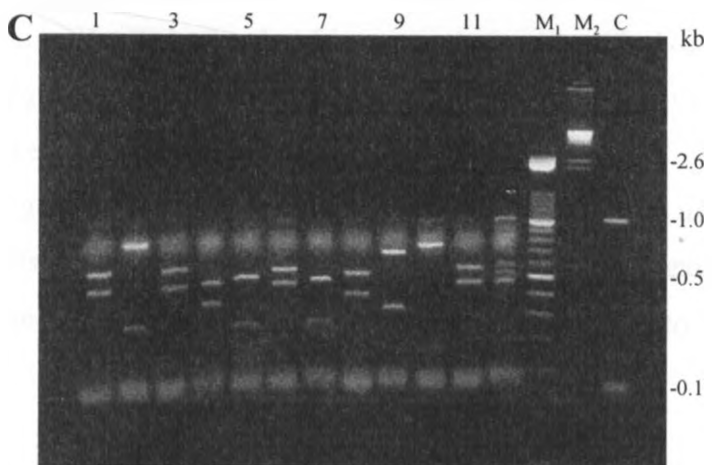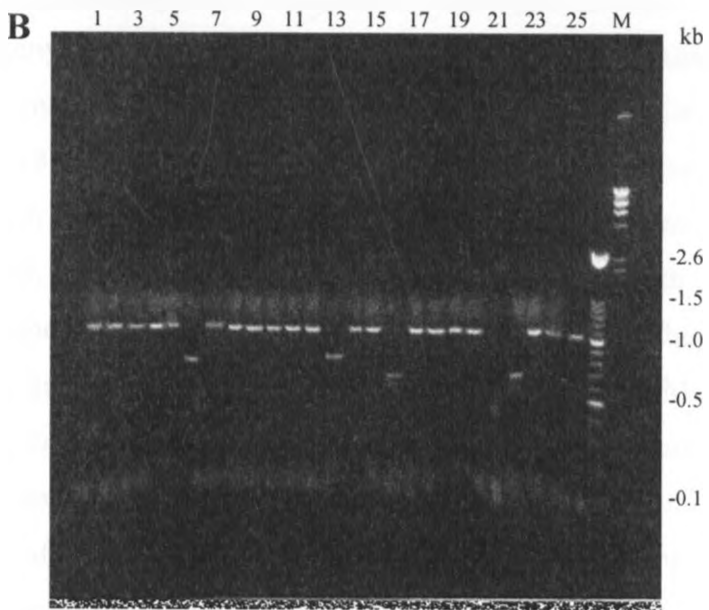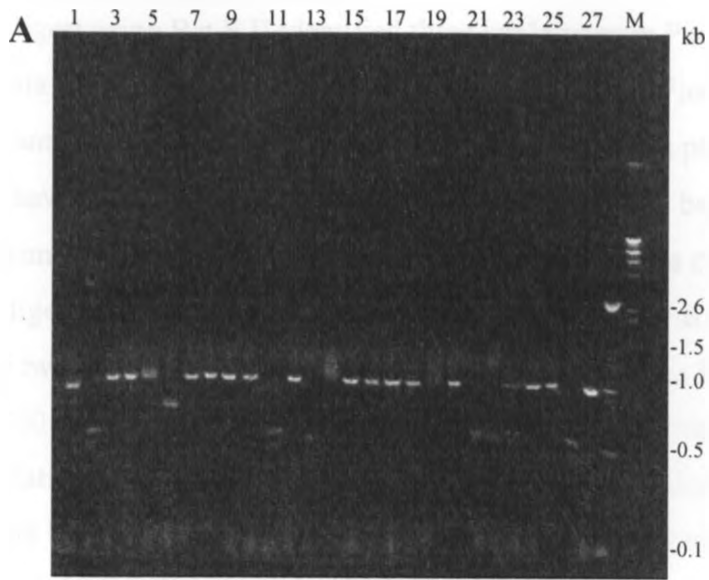
3.6.2. Restriction Fragment Length patterns

Restriction digest using Bst E II identified three haplotypes in Pemba, Australia (Murlimbugah), Costa Rica (Mag Diamanted), Zanzibar, Costa Rica (Finca Guadaloupe), Australia (Coffs), Tanzania, Columbia and Puerto Rico (fig. 24a). It is possible that all the ten populations have two restriction sites which cleave the 1000 bp band to give three fragments of approximately 250 bp, 500 bp and 750 bp, even if enzyme concentration or the duration of the digest was increased to rule out incomplete digests. It is likely that the enzyme cuts to give two equal fragments of approximately 250 bp and a fragment of about 500 bp. The750 bp fragment is likely to be as a result of incomplete digestion. The rest of the populations: Thailand, South Africa, Mbita, Honduras, Embu, Cameroon, Uganda (Masaka) and Uganda (Kawanda) all have a restriction site and cleaves the 1000 bp product to give only two fragments of 250 bp and 750 bp.

Restriction digests using Hae III identified 4 haplotypes in Pemba, Mbita (Kenya), Honduras, Embu (Kenya), Costa Rica Md, Zanzibar, Costa Rica FG, Australia (Coffs), Ghana, Tanzania, Cameroon, Uganda (Masaka), Uganda (Kawanda), Columbia and Puerto Rico (Figure 18b). These populations indicate the presence of three or more restriction sites, which cleave the 1000 bp product to give four fragments of approximately 100 bp, 150 bp, 200 bp and 500 bp. The fragment length of the different profiles for this enzyme do not add up to 1000 bp, this is attributed to the presence of additional small fragments that are not resolved on the gel. Australia (Murlimbugah) only showed two haplotyes indicating possible presence of two restriction sites, which cleave the 1000 bp into two fragments of 250 bp and a 500 bp fragment. Two haplotypes were identified in Thailand population indicating the presence of more than 1 restriction site, which cleaves the 1000 bp to give two fragments of approximately 250 bp and 600 bp and other small fragments that are not resolved by the gel system used. Restriction digests using HaeIII in South African population identified only one haplotype. This indicates the presence of one restriction site which cleaves 1000 bp fragment to give two equal fragments of 500 bp (figure 24b).

Restriction digests of the 1000 bp product in all the populations using Dde I showed two fragments in all the populations of approximately 350 bp and 500 bp. The fragment length of the different profiles for this enzyme do not add up to 1000 bp, this is

attributed to the presence of additional small fragments that are not resolved on the gel system (figure 24c). Restriction digests of the 1000 bp product in all the populations using Hinf I showed four fragments in all the populations of approximately 200 bp, 250 bp, 500 bp and 750 bp (figure 24d). The sum of the lengths of the bands even after several repeats of this experiments consistently exceeded the length of the amplicon. Although these bands appeared to be caused by incomplete digests they could not be eliminated by increasing enzyme concentration or the duration of digest. As a result of difficulties in interpretation this data was not discussed.

Restriction digests of 1000 bp product in all the populations using Hpa II identified two fragments of 400 bp and 500 bp. The fragment length of the different profiles for this enzyme do not add up to 1000 bp. This is attributed to the presence of additional small fragments that are not resolved on the gel system used (figure 24e). Restriction digest of 1000 bp product in all the populations using Nde II identified two fragments of 700 bp and 300 bp (figure 24f). From the RFLP of Nde II digests, the location of Nde II sequence recognition site appears to be conserved among the populations studied.

Restriction digests of 1000 bp product in all the populations except Thailand using RSA I identified two fragments of 150 bp and 750 bp. The fragment length of the different profiles for this enzyme do not add up to 1000 bp. This is attributed to the presence of additional small fragments that are not resolved on the gel system used (figure 24g). Restriction of the 1000 bp product in Thailand population with RSA I gave two fragments of 150 bp and 650 bp. There is a possibility of three or more restriction sites being present that give rise to fragments of 150 bp and 650 bp fragment. This fragment lengths do not add up to 1000 bp. This is attributed to the presence of additional small fragments that are not resolved on the gel system used (figure 24g).

The restriction of all the 100 bp products in all the populations using Xba I identified two fragments of approximately 450 bp and 550 bp (figure 24h). The Xba I sequence recognition site appears to be conserved amongst the populations studied. Some enzymes revealed non-variant (non-polymorphic recognition sites) in the different weevil populations studied. Such restriction enzymes include Hinf I, Dde I, Hpa II, Nde II and Xba I. Polymorphism was observed with respect to the location of BstE II site.

BstE II had one recognition site giving two fragments of 250 bp and 750 bp in nine populations and two recognition sites giving three fragments of 250 bp, 500 bp and 750 bp in the rest of the nine populations (table 21). Restriction fragments for Columbia and Puerto Rico samples are shown in figs. 24i and j.

Hae III gave four fragments in sixteen of the populations indicating the presence of three recognition sites. Australia (Murlimbugah) had two sites giving two equal fragments of 250 bp each and 500 bp. Thailand population gave a unique fragment of approximately 600 bp. South Africa seems to have only one recognition site giving two equal fragments of 500 bp each. RSA I gave a unique fragment of 650 bp with Thailand population. Alu I restricted the 400 bp in Pemba samples to give a fragment of 200 bp. It gave what seems to be partial digests in all the other populations (fig. 25).

Figure 24:

1.5 % agarose gels showing restriction fragments digests of banana weevil mitochondrial CO1 1000 bp amplicons. Samples 1-17 are in the order indicated in section 4.8.1 for all cases from A-H.

A- Bst E II digests

B- Hae III digests

C- Dde I digests

D- Hinf I digests

E- Hpa II digests

F- Nde II digests

G- Rsa I digest

H- Xba I digests

I- Columbian samples digested with Ava II, BstE II, Dra II, Hae III, Dde I, Hinf I, Hind II, Hpa II, Nde II, Rsa I, Sau I and Xba I

J- Puerto Rico samples digested with Ava II, BstE II, Dra II, Hae III, Dde I, Hinf I, Hind II, Hpa II, Nde II, Rsa I and Xba I

I

| 1 | 3 | 5 | 7 | 9 | 11 | M | kb |

-2.6
-1.5
-1.0
-05
-03
-01

J

| 1 | 3 | 5 | 7 | 9 | 11 M | kb |

-2.6
-1.0
-05
-03
-01
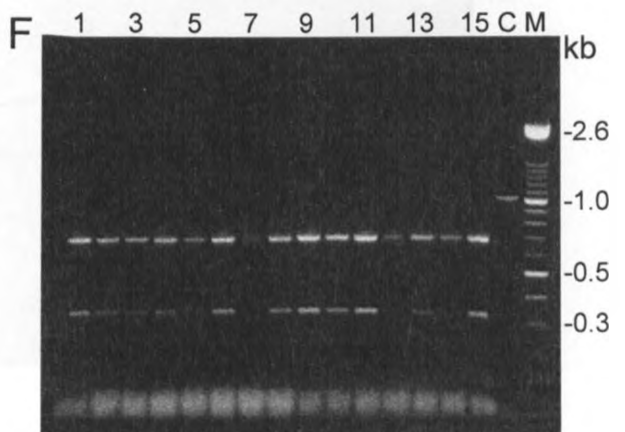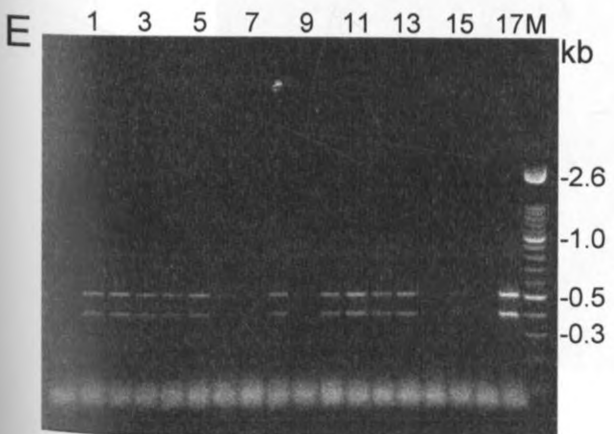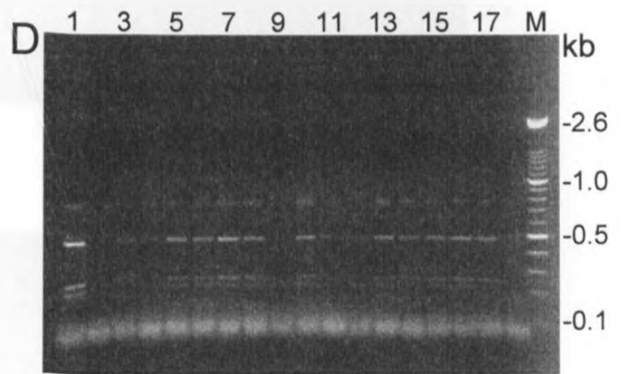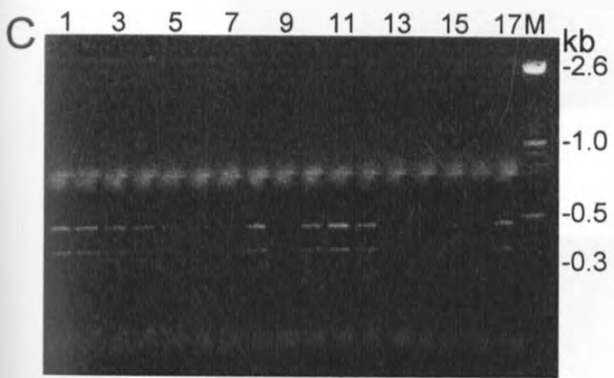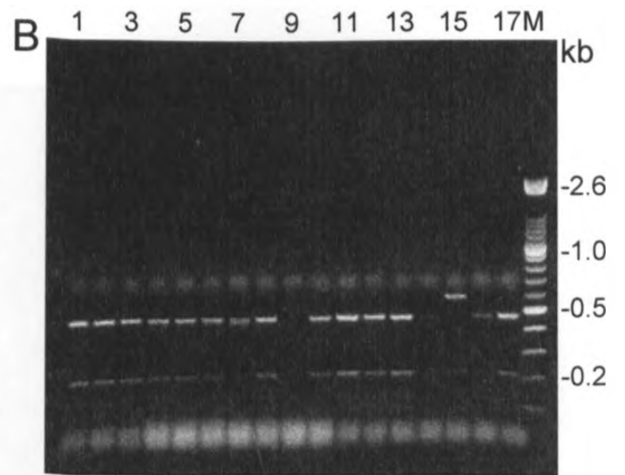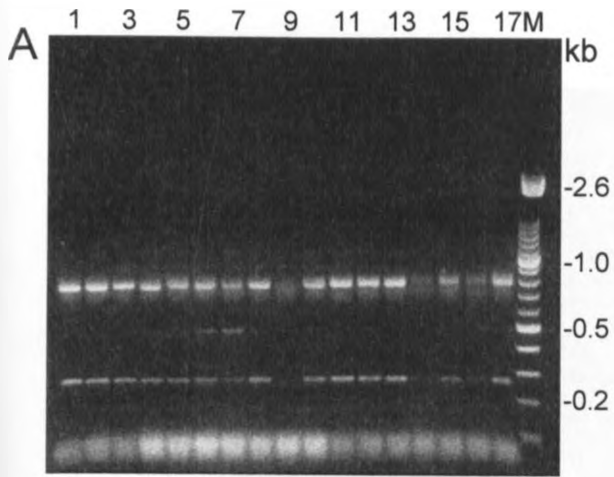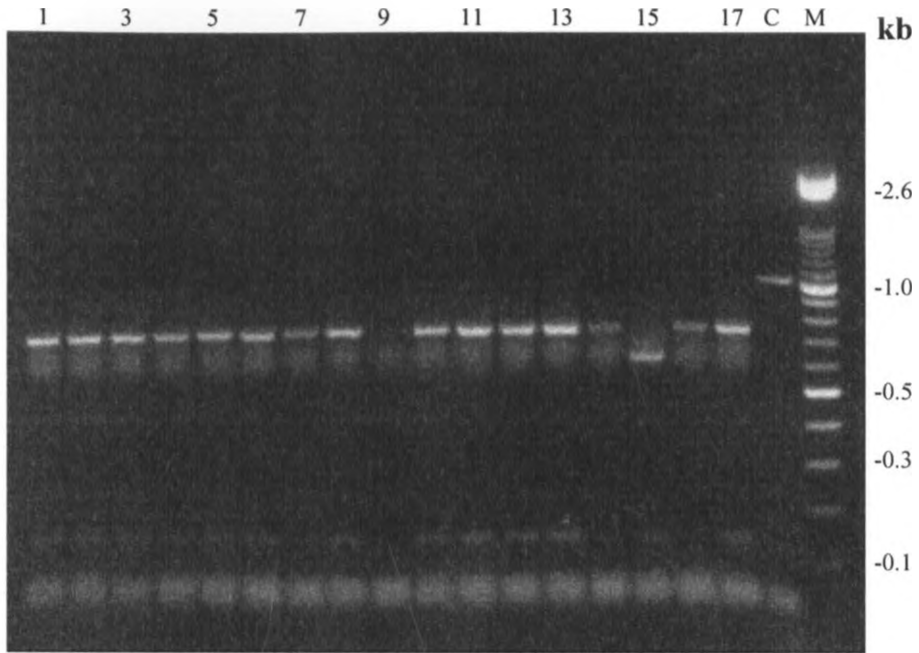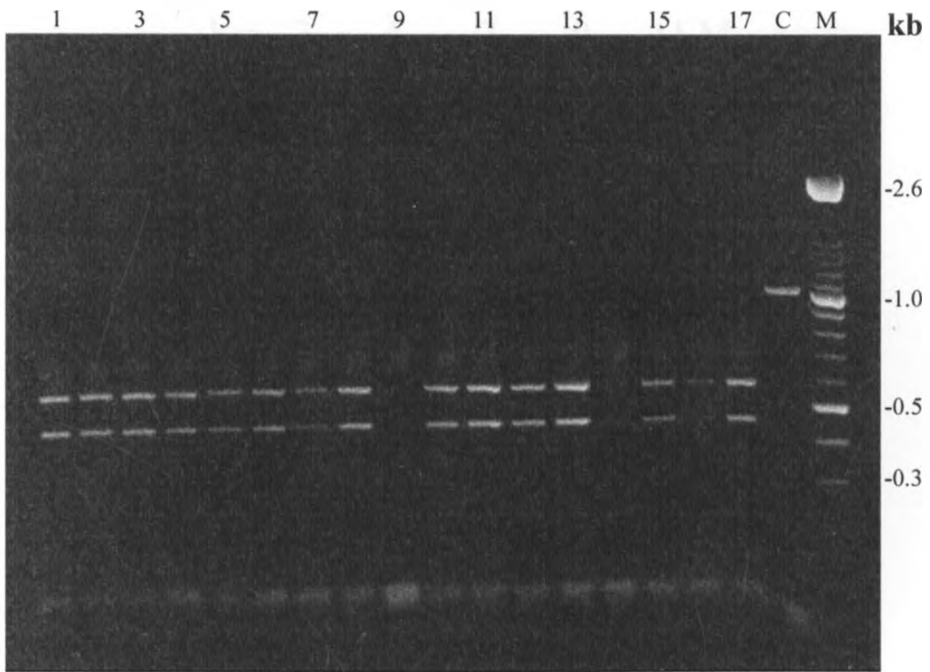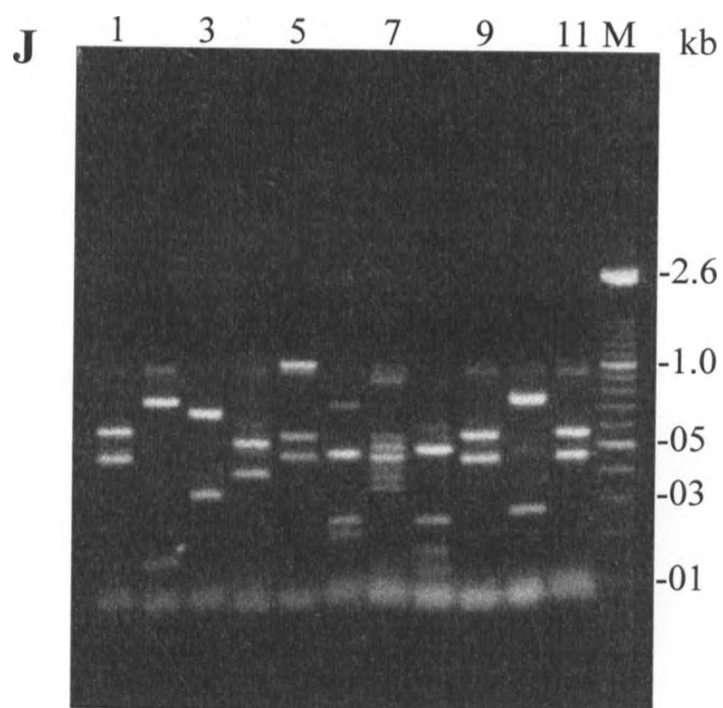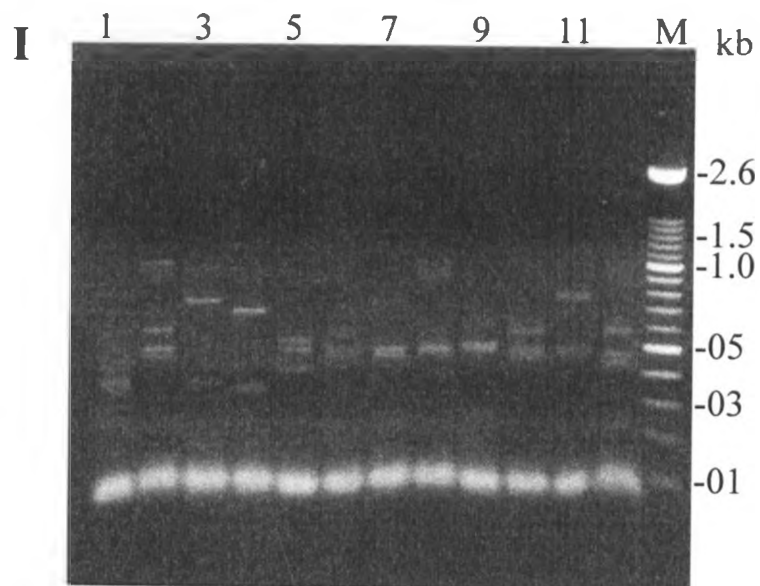
Figure 25:

A 1.5 % agarose gel showing restriction digests of banana weevil [1 mitochondrial CO1 400 bp amplicon using Alu I. Samples 1-17 a are in the order indicated in section 4.8.1. Puerto Rico samples are not incl fluded.
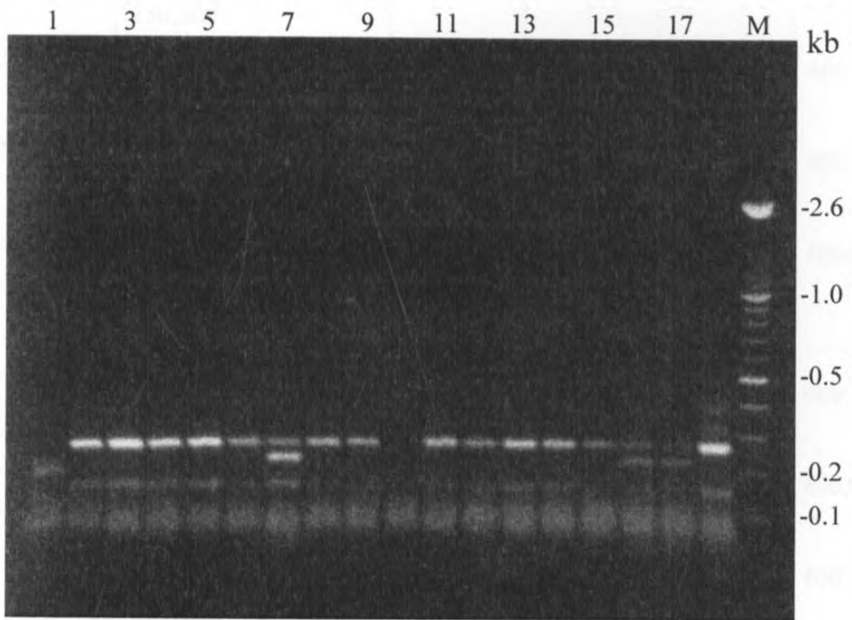
Table 21: Restriction Fragment lengths estimated on 1.5% agarose gel for geographical population of *Cosmopolites sordidus* for the 1000 bp amplicon

Geographic source	BstEII	HaeIII	dde	HpaII	NdeII	RSAI	XbaI	HinfI
Pemba	250,500 750	100,150 250[a],500	350,500	400,500	300,700	150,750	400,600	200,250 500,750
Australia (Murlimbugah)	250,500 750	250,500	350,500	400,500	300,700	150,750	400,600	200,250 500,750
Thailand	250,750	250,650	350,500	400,500	300,700	150,650	400,600	200,250 500,750
SouthAfrica	250,750	500[a]	350,500	400,500	300,700	150,750	400,600	200,250 500,750
Kenya (Mbita)	250,750	100,150 250,500	350,500	400,500	300,700	150,750	400,600	200,250 500,750
Honduras	250,750	100,150 250,500	350,500	400,500	300,700	150,750	400,600	200,250 500,750
Kenya (Embu)	200,750	100,150 250,500	350,500	400,500	300,700	150,750	400,600	200,250 500,750
Costa Rica (Md)	250,500 750	100,150 250,500	350,500	400,500	300,700	150,750	400,600	200,250 500,750
Indonesia	-------	--------	--------	-------	--------	--------	---------	---------
Zanzibar	250,500 750	100,150 250,500	350,500	400,500	300,700	150,750	400,600	200,250 500,750
Costa Rica (FG)	250,500 750	100,150 250,500	350,500	400,500	300,700	150,750	400,600	200,250 500,750
Australia (coffs)	250,500 750	100,150 250,500	350,500	400,500	300,700	150,750	400,600	200,250 500,750
Ghana	250,750	100,150 250,500	350,500	400,500	300,700	150,750	400,600	200,250 500,750
Tanzania	250,500 750	100,150 250,500	350,500	400,500	300,700	150,750	400,600	200,250 500,750
Cameroon	250,750	100,150 250,500	350,500	400,500	300,700	150,750	400,600	200,250 500,750
Uganda (Masaka)	250,750	100,150 250,500	350,500	400,500	300,700	150,750	400,600	200,250 500,750
Uganda (Kawanda)	250,500 750	100,150 250,500	350,500	400,500	300,700	150,750	400,600	200,250 500,750
Columbia	200,500	100,500	350,500	400,500	300,700	150,750	400,600	200,250

Puerto Rico	750 250,500 750	250,500 100,150 250,500	350,500	400,500	300,700	150,750	400,600	500,750 200,250 500,750

CHAPTER FOUR

4.0 DISCUSSION

4.1 Genenetic diversity in banana weevil

4.1.1 Weevils from different sites of the world

Three measures were used to quantify the amount of genetic variability as shown in section 1.9.4.1. In this study, because of the large number of polymorphic loci revealed within individuals by arbitrarily primed PCR, the data can be used to address intraspecific questions concerning the genetic relatedness of individuals as well as populations of banana weevil. The use of RAPD analysis has allowed individual *Cosmopolites sordidus* to be characterized on the basis of their distinct electrophoretic types permiting closely related individuals to be readily distinguished from each other. Little is known about the genetics and the population structure of this species. The results show that under the described conditions, individual RAPD phenotypes were highly reproducible. This is the first RAPD report within and between natural populations of *C. sordidus*. Analysis of these RAPD data using suitable numerical methods has provided an indication of the genetic heterogeneity of *Cosmopolites sordidus* from different parts of the world, therefore RAPD fingerprinting appears to be valuable for population studies of the banana weevil. Likelihood ratio (G^2) test for overall genetic structure was significant for 27 out of 46 indicating that RAPD divergence among natural populations of banana weevil was due mainly to band frequency differences rather than the fixation of locally common or rare bands.

The percentage of polymorphic loci clearly demonstrated genetic diversity in the banana weevil. 92.2 % polymorphism was estimated for world samples. Primer OPO 14 was on average polymorphic at 90 % of the loci. OPA17, OPO18, OPO19 and OPO20 were on average polymorphic at 96 %, 94 %, 85.3 % and 95.6 % of the loci respectively. The observed differences in RAPD polymorphisms may simply reflect different abilities of the different primers to detect variation. For the world populations, the Mbita (Kenya), PuertoRico and Tanzania populations had the highest absolute level of polymorphism (45 out of 46), while the Honduras population had the lowest (36 out of 46). The diversity in Banana weevil from different regions of the world revealed by amplification of total

cellular DNA with random primers revealed low to moderate variabililty in different weevils populations world wide. The average genetic heterozygosity among RAPD loci (H_t=0.470) was within the range reported in most insects i.e. within species genetic distances (Nei 1978) unbiased genetic distances of 0.4, 0.5 and 0.76 for *M. raptor* form North Carolina, Utah and Israel (Taylor *et al.*, 1997). The range of within population distaces of (0.13-0.57) were observed in the mosquito (Ballinger-Crabtree, 1992). Estimates of Shannon's phenotypic diversity (H_o) for RAPD ranged from 0.382 (Honduras) to 0.806 (Florida) and the mean for the 15 populations was 0.623 indicating genetic variability in the banana weevil. Examination of genetic diversity present within populations, (H_{pop}/H_{sp}), and between populations, ($H_{sp}-H_{pop})/H_{sp}$ indicated that on the average the diversity (74.7 %) occurred within weevil populations.

The amount and partitioning of genetic variation among and within populations results from the dynamic processes of gene flow, selection, inbreeding, genetic drift and mutation (Hartle and Clark, 1994). Thus knowledge of the current genetic structure allows inferences about past processes. At the same time genetic variation represents the starting point for further evolution and is an important prerequisite for the prediction of evolutionary responses. This is of practical significance in the light of human-caused habitat fragmentation, alteration or destruction during banana farming.

A high level of genetic variation within populations (74.7 %) was observed in this study and a relatively srong level of differentiation between geographical populations. *C.sordidus* is directly affected through human manipulation of the environment. Breeding sites are created as a result of banana farming activities and with the increased transportation of infected planting material. Human activities associated with the movement of planting material encourage the spread of this species favouring outbreeding between populations and promoting a certain degree of genetic homogeneity. This kind of homogeneity was especially evident between the sampling areas, Tanzania, Mbita and Zanzibar which despite being separate by several km from Columbia shared genetic affinities with Columbia. With the struggle for planting materials that are resistant to disease, pests and high yields, a large number of planting material movement occurs to all parts of the banana growing regions of the world promoting weevil spread. The effects of small population size with accompanying genetic drift represent an

important component to understanding genetic variation in natural populations of banana weevils because of control efforts by humans. Unlike positive assortative mating, which only affects a single locus, small population size and drift affect the entire genome, including those traits that are important to vectorial capacity and competence as observed in arthropod vectors of disease (Hartl and Clark, 1994). Banana weevils, which are serious pests are different from many other organisms because they are often directly subject to human efforts to control populations. The efforts to reduce populations through pest control play a major role in changing gene frequencies through genetic drift.

The mutation of alleles into variant forms is the ultimate source of new genetic material. The effect on genetic variation is dependent on the mutation rate. Mutation rates alone are not believed to be high enough to play a major role in the population genetics of a species (Tabachnick and Black IV, 1996). However, mutation rates in noncoding regions, especially in highly repetitive sequences with simple repeat structures are known to be greater, and mutation rates cannot be ignored when using these in population studies. Mutation rates in these regions are probably greater because they do not code for a gene product and are, therefore, not constrained by selection. However if they occur as tandem repeats, specfic molecular events can cause them to mutate at greater rate. The rate with which selection can act is proportional to the fitness differences between genotypes, and such differences are dependent on environment in which the population is found. RAPD and RFLP have a measurable effect on fitness (Tabachnick and Black IV, 1996). They are considered to be neutral in their impact on fitness, and variation in their frequencies among weevils populations is most likely caused by low levels of migration (mainly through planting material), mutation, population size, and drift.

It is suggested that two factors may have caused the greater differentiation among populations. Firstly, weevil dispersal may be a limiting factor for gene flow. Secondly the wide separation between populations may also result in increased genetic diversity. The populations sampled in this study were separated by many kilometers and this distance could be a significant factor accounting for the observed genetic diversity between populations. Thus the observed values (in range of 0.85 – 0.96) for worldwide

populations 0.99) isvery close to the limit usually recognized for genetic identities between species of 0.85 – 0.90 (Crawford, 1983).

4.1.2 Banana weevils from different sites in Uganda

For the samples that were collected from different regions of Uganda, the average heterozygosity (Ht) among RAPD loci was 0.46 indicating a reasonable amount of diversity. 94.2% polymorphism was estimated. Estimates of shannon's phenotypic diversity (H_o) for RAPD ranged from 0.491 to 0.77 averaging 0.619 for the 15 populations indicating a lower diversity in comparison to world populations. Examination of genetic diversity present within populations, (H_{pop}/H_{sp}), and between populations, (H_{sp}-H_{pop})/H_{sp} indicated that on the average the diversity 84.7% occurred within weevil populations.

4.2 Molecular markers for banana weevil from different sites of the world

Specific molecular markers for the different clusters were identified. The second cluster (made up of Puerto Rico) was characterized by a 500 base pair fragment generated by primer OPO 14. OPA 17 conistently gave a 500 base pair fragment with the first cluster. (Columbia, Zanzibar, Mbita-Kenya and Tanzania). OPO 18 on the other hand amplified a 400 bp fragment in the sixth cluster (Honduras, Costa Rica and South Africa). This proved the distinctiveness of the clusters.

4.3 Population structure of the banana weevil

4.3.1 Weevils from different parts of the world

The results of the study clearly showed genetic variability in the banana weevil. The populations were found to fall into six clusters depending on their degree of similarity. Columbia, Zanzibar, Mbita-Kenya and Tanzania clustered together in the first cluster. In the second was Puerto Rico, Indonesia, Australia and Florida in the third; Ghana, Embu and Uganda clustered together in the fourth; Cameroon alone made up the fifth, and in the sixth were Honduras, Costa Rica and South Africa.

4.3.2 Weevils from different parts of Uganda

The Ugandan populations were found to fall into 9 clusters. Kalegueri, Namubiru in the first; Bukanga in the second, whereas Kabukye and Bundagara grouped together in a third cluster. Fourth cluster was made up of Namunyonyi, Kawanda and Ntinkalu in the fifth, Ikuluwe and Lake shores made up the sixth, while in the seventh was Ntengeru. The eighth was made up of Bunyaruguru and in the ninth were Buwama and Buzaya.

4.4 Genetic differentiation in the banana weevil

4.4.1 Banana weevils from different parts of the world

Overall Gst was 0.13 indicating lower differentiation among the populations. Overall Gst given by DISPAN was equal to 0.216, indicating strong differentiation among populations. This implies that approximately 22 % of total genetic variability observed in this study was due to differentiation between populations compared with 78 % within populations. The smallest Gst values were found in Tanzania and Mbita (Kenya) (0.0379), Zanzibar and Mbita (0.0669) and Tanzania and Columbia (0.1006). Highest Gst value was found between Embu (Kenya) and Indonesia (0.5525). Higher Gst values were in Indonesia and Ghana (0.5110), Indonesia and Honduras (0.4718), Embu (Kenya) and Florida (0.4011) and Embu (Kenya) and Australia (0.3924). Indonesia and Kenya (Embu); Indonesia and Ghana; Indonesia and Honduras had Gst values higher than 0.4 in the case of world populations. All of the interpopulation distances were lower than 0.4 (which is the grand mean Nei 1972) distance for populations of congeneric species (Crawford 1989). Most mean interpopulation Nei (1972) distances higher than 0.4 involve Indonesia samples, while all the other interpopulation distances are lower than 0.4.

We can thus hypothesize that the first step in banana weevil evolution was the diversification of Indonesia populations which appear primitive, since the genetic distances between Indonesia and Kenya (Embu), Indonesia and Honduras and Indonesia and Ghana were as high as those usually reported between different species (Crawford 1989).

The second step would have involved the diversification of the other populations which appear to have evolved more recently, since the genetic distances between them are lower than those usually reported between different species (Crawford 1989).

Estimates of gene flow (Nm) ranged from 0.617 to 1.05, averaging 0.811 within populations in this study. This indicated extremely rare migratory events or no migration at all. According to Crow and Aoki (1984), Nm values of less than 1 indicates little or no gene flow. This is expected in this study considering the geographic isolation of these populations and the fact that migration is minimal and is mainly through planting material. Thus isolation by distance and minimal dispersal might have been an important factor in the genetic differentiation of the banana weevil. If migration occurs between two populations, they always share some common alleles and differentiation is hindered. When 2 populations differ in gene frequencies and exchange migrants, the gene frequencies in each population change depending on the proportion of migrant individuals. However, as a result of artificial dispersal of weevils by humans through transportation of infested planting materials in the last few hundred years, weevils are now found in all banana growing regions worldwide. During this distribution, populations of the species may have experienced numerous genetic bottlenecks and periods of isolation and geographically isolated populations became genetically distinct. Many weevil populations probably occur with population sizes that because of seasonal fluctuations such as heat, freezing and desiccation, or control efforts become small. At these times, random chance and genetic drift can have significant consequences on gene frequencies. *Ae. Aegypti* populations, particularly on many Caribbean islands, demonstrated this effect. Islands such as Jamaica, Puerto Rico, Martinique, Barbados, and Aruba have extensive mosquitoe control activities directed against *Ae. Aegypti*. These control activities cause reduced mosquitoe population size. However, mosquitoe population sizes fluctuate because control activities are often discontinued until the mosquitoes become abundant (Tabachnick and Black IV, 1996).

This implies that approximately 13% of total genetic variability observed in this study was due to differentiation between populations compared to 87% within populations.

4.4.2 Banana weevils from different parts of Uganda

The smallest Gst value was found between Ikurwe and Lakeshores (-0.008) indicating that the two populations are almost 100% similar. This is because the two locations are next to each other and there is a possibility of a lot of weevils from one location migrating to the other and vice versa, leading to genetic homogeneity. While the highest Gst value was observed between Bunyaruguru and Buwama (0.1779). Estimates of gene flow (Nm) ranged from 1.305 – 1.553. Over all populations, according to Crow and Aoki (1984), gene flow values (Nm) of less than 1 should be interpreted as little or no gene flow. Thus, the average of the values (Nm = 1.45) observed within populations in this study would indicate reasonable migratory events. This clearly explains the low level of Gst observed. If migrations occurs within two populations, they always share some common alleles and differentiation is hindered. Due to geographic proximity of all the sites studied in Uganda, there is higher chances of dissemination of banana weevils from one location to another through infested planting material. There could even be possibility of crawling from one site to another. Torrential downpours of rain may also carry infested plant material and weevils in the soil from high to low levels. This could possibly explain the low differentiation and low variability observed almost tending towards homogeneity.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of the CO1 mitochondrial region was used to study variation in the weevil. Low variability was observed. Owing to its mode of inheritance, mtDNA is more sensitive to bottlenecks in population size and to population subdivision than are nuclear genes. Joint comparative studies of both mtDNA and nuclear DNA variability give valuable insights into how effective population size has varied through time. Such studies also give insight into the conditions under which mtDNA from one population can colonize another.

4.5 PCR-RFLPs within the COI mitochondrial region of banana weevil from different sites of the world

There was no amplification using the CO1 mitochondrial primers in all the samples from Indonesia, indicating how genetically remote the Indonesian samples are from the rest of

the samples. This confirms the hypothesis derived from RAPD data. It appears as though Indonesian populations develop more independently than the other weevil populations. Perhaps it has been genetically isolated from other populations for longer, during which time genetic drift has increased its differentiation. Digestion of the CO1 amplicon (1000 bp) with Bst E II restriction enzyme produced patterns that were interpretable on agarose gels. It distinguished the populations into two groups just like it was done by RAPD at 85 % similarity level, except for Mbita (Kenya) population. Pemba, Australia (M), Costa Rica (Md), Zanzibar, Costa Rica (FG), Australia Coffs, Tanzania, Columbia and Puerto Rico formed the first group. The second group that had two fragments included Thailand, South Africa, Mbita (Kenya), Honduras, Embu (Kenya), Cameroon, Uganda (Masaka) and Uganda (Kawanda).

Restriction enzyme Hae III distinguished Pemba, (Mbita) Kenya, Honduras, Embu (Kenya), Costa Rica (Md), Zanzibar, Costa Rica (FG), Australia (Coffs), Ghana, Tanzania, Cameroon, Uganda (Kawanda), Columbia and Puerto Rico from Australia (Murlimbugah) andThailand populations, which had unique restriction patterns from all the the rest of the populations.

Dde I did not give any useful patterns to distinguish the populations. Hinf I consistently gave partial digests, which obscured interpretation of RFLP patterns. Hinf I restriction digest for *C. sordidus* had faint bands in addition to the stronger bands, and the sum of the bands consistently exceeded the length of the initial amplicon. Although these bands appeared to be caused by incomplete digests, they could not be eliminated by increasing enzyme concentration or duration of digest. Because of the difficulty in the interpretation, it was not discussed. A similar observation was made by Taylor *et al.*, (1997) in wasps in the genus *Muscidifurax*. Hpa II, Nde II and Xba I identified fragments that were similar in all the populations and hence were not useful in the study of genetic variation in the weevil.

Digestion of the CO1 amplicon with eight commercially available restriction enzymes produced patterns that were interpretable in agarose gels, although only four of the eight tested enzymes proved useful. Hinf I consistently gave partial digests, which obscurred interpretation of RFLP patterns. Four restriction enzymes, BstE II, Hae III, Alu I and Rsa I resulted in digest patterns which were diagnostic among the weevil

populations studied. Differences between the fragment patterns are usually due to base substitutions that cause restriction sites to be gained or lost but, sometimes are the result of length mutation (Brown and DesRosiers, 1983; Cann and Wilson, 1983; Ferris *et al.*, 1983c; Greenberg *et al.*, 1983; Cann *et al.*, 1984). Because a single breeding pair of diploid animals contain four nuclear genomes and one transmissible mtDNA, a population that goes through an extreme bottle neck could lose all of its mtDNA variability and retain a significant fraction of its nuclear variability (Nei et al., 1975; Barton and Charlesworth, 1984). In this study, there were reasonable differences among the various populations as regards nuclear genes, nevertheless there was remarkable homogeneity with low variability among the weevil populations with respect to their mtDNAs. The times at which these apparent bottlenecks took place are in most instances probably very recent and suspected to be due to human influence.

Kambhampata and Rai (1991) examined RFLP variation among 17 worldwide populations of *Ae. Albopictus* and found 99% of fragments were shared among populations. Mitchell *et al.*, (1992) found similarly low variability in mtDNA RFLPs in the four sibling species of the *An.quadrimaculatus* species group. In this study low mtDNA RFLP was also observed in the banana weevil. This low variability suggests either that populations were too recent in origin to have accumulated differences or that the mtDNA in *C. sordidus* evolves slowly.

Conclusion

In conclusion, the results of the study clearly showed low to moderate variability in different weevil populations worldwide. Indeed, the project hypothesis was that genetic variability might be expected in these weevils given their restricted mobility and monophagy. The populations were found to fall into several distinctive clusters depending on their degrees of genetic similarities. In addition, specific molecular markers for the different clusters were identified. This proved the genetic distinctiveness of the clusters. In another study, the genetic variability in weevil populations was analyzed in samples obtained from 15 sites in banana growing regions of Uganda. Although the weevil populations in this case were very similar genetically, some variability was observed.

In the present study, conclusions drawn from RAPD analysis were supported by CO1 mtDNA, PCR-RFLPs data. mtDNA showed low variability and diagnostic fragments that could be used to differentiate certain populations. It appears that sufficient divergence exists in these mtDNA region for a large scale assessment of diagnostic polymorphism. Banana weevil relationships cannot be determined based only on the limited nucleotide data presented here. Yet there is enough sequence divergence in the mitochondrial region to suggest that an expanded taxonomic survey would be phylogenetically informative.

It is suggested that a number of factors may have caused the genetic differentiation among these populations. Wide separation between populations may have resulted in genetic distance observed. It is clear from the study that genetic relatedness and geographic proximity are not directly related in all cases in the study as is the case in where East Africa share genetic affinity with Columbia. The other factors include length of time in different regions, generation time, reproductive levels, genetic drift, environmental factors (elevation, rainfall, soil type), host (type of banana), stress – related (entomopathogenic fungi, pesticides) and selection pressures.

The existence of weevil biotypes as shown by the study suggests the need for multilocation testing with respect to resistant germplasm, to different strains of fungal pathogens, entomopathogenic nematodes or to predatory – prey studies with weevil natural enemies especially in weevil populations with high levels of variability loike Ghana and Indonesia.

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APPENDIX

I   BACTERIAL MEDIA AND PLATES:

2x YT (per litre):

16g bactotryptone

10g yeast extract

5g Nacl

Ampicillin was dissolved at a concentration of 100mg/ml and stored at −20°C. When required ampicillin was added to the media at a concentration of 50µg/ml.

NZCYM medium for plates (per litre):

10g NZ amine.

5g Nacl.

1g casamino acids.

1.71g $MgSO_4.7H_2O$

2.06g Agar

II   BUFFERS AND SOLUTIONS

CTAB extration buffer:

50mM Tris pH 8.0

10mM EDTA

0.7M Nacl

1.0% Cetyl-trimethyl ammonium bromide (CTAB)

0.1% β-mercaptoethanol

TE Buffer

10mM Tris-HCL pH 8.0

1mM EDTA

Cell suspension solution

500mM Tris-HCL pH 8.0

10mM EDTA

100µg/ml RNAse A

Cell lysis solution

0.2M NaOH

1% SDS

Neutralization buffer (For miniprep)

2.55M potassium acetate pH 4.8

Column wash solution

200mM Nacl

Tris-HCL, pH 8.0

5mM EDTA

Diluted 1:1 with absolute ethanol.

Denaturation solution

0.5N NaOH

1.5M Nacl

Neutralization solution (For southern transfer)

0.5M Tris-HCL, pH 7.5

3M Nacl

20x SSC stock solution

3M Nacl

0.3m sodium citrate, pH 7.0 ($+20^{0}$C), autoclaved)

N-Lauroylsarcosine stock solution

10% (w/v) in H_2O

Filtered through a 0.2-0.45 µm membrane.

SDS stock solution

10% (w/v) in H_2O

Filtered through a 0.2 0.45 µm membrane.

Blocking reagent stock solution

Blocking reagent is dissolved in maleic acid buffer to a final concentration of 10% (w/v) with shaking and heating.

Standard hybridization buffer

5x SSC

0.1% N-lauroylsarcosine

0.02% SDS

1% blocking reagent

Blocking buffer

Dilute blocking reagent stock solution 1:10 with Maleic acid buffer.

Washing solution 2x

2x SSC

0.1% SDS

Maleic acid buffer

0.1M maleic acid

0.15 Nacl pH 7.5 (+20^{0}C).

Adjust pH with concentrated or solid NaOH, autoclave

III. RAPD band scoring sheet for Banana weevils from different geographic locations

O14-01 O14-02 O14-03 O14-04 O14-05 O14-06 O14-07 O14-08 O14-09 A17-01 A17-02 A17-03 A17-04 A17-05 A17-06 A17-07 A17-08 A17-09 O18-01 O18-02 O18-03 O18-04 O18-05 O18-06 O18-07 O18-08 O18-09 O19-01 O19-02 O19-03 O19-04 O19-05 O19-06 O19-07 O19-08 O19-09 O19-10 O20-01 O20-02 O20-03 O20-04 O20-05 O20-06 O20-07 O20-08 O20-09

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Australia 01 110011011100111001101010111010101010001111010
Australia 02 110011100100110100111110000011101110011111110
Australia 03 110011001101111100011111000000110110101111110
Australia 04 100111100100101100011000000001101101000101000000
Australia 05 110111010100111100011110000011101110011111110
Australia 06 101000000000011110010000100110101000100100100
Australia 07 000010010000000000000000000000101000001001100
Australia 08 110011001101111100011100001000001111011011110
Australia 09 100111001110101100111000000000011101111111010
Australia 10 110001001000011100011000000000101000001110101
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III.RAPD band scoring sheet for Banana weevils from different geographic locations

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Indonesia 22 11010110011111100101000001000000000111010100
Indonesia 23 110111000111101101101000000111000111100
Indonesia 24 111111000111110'0011000100101001000110100000
Indonesia 25 11100010011111910000000000010000010101001000
Indonesia 26 11111000010110130111001000011011001001010100
Indonesia 27 11111000011111010000000000000000000000111100000

Indonesia 28 000000000000000000001110000001001100101001100010
Indonesia 29 110011000111110000010000000110000110000101001
Indonesia 30 11111000011110101001100000011111100001111010110
Indonesia 31 11111000000000000111100000110111011010111101000
Indonesia 32 110110000000000000110110000000000000101101101
Indonesia 33 11101010001111000011110000111110000011111100
Indonesia 34 11111110011111101101010000011111111101111111000
Indonesia 35 111111001101111010010000001000010000111111000
Indonesia 36 111110000111111001110000001000100001111111000
Indonesia 37 001100000111111001111100001100100000011000001
Indonesia 38 1100000011111110001100000000000010000000000000
Indonesia 39 100000000111110000000000000001000011001110000100
Indonesia 40 1110100001110010001100100011110001001110100000
Indonesia 41 110001000000000001011000000000000000000000000
Indonesia 42 11111110000000000000000000001100101000101000000
Mbita 01 11111111100000000001111111000000000011111111
Mbita 02 1111101011110100001100111110111111000000000
Mbita 03 11111010100000110111000101010111110000000000
Mbita 04 0000000001111001000011001000000000000000000
Mbita 05 000000000010010010011111101011111111111111
Mbita 06 00000000011000100111001110000000000000111010
Mbita 07 00000000011111100111111110111111111111111
Mbita 08 110111000111101100011111111001111111111111
Mbita 09 111111110111111001110000000000000011111000
Mbita 10 00000000111111010011011010101111111111111111
Mbita 11 111111000000000001011111000000000001111111
Mbita 12 1111101011011001111001010111111111111111
Mbita 13 111111101111100001111101000111111111111111
Mbita 14 111111110111111001111101100000000001111111
Mbita 15 11111110110100001010000101111111110111111
Mbita 16 111111110101010011111010011111111111111111
Mbita 17 111111111111011001101011010111111111111111
Mbita 18 11111111111111000111100010011011111111111
Mbita 19 1111111111110000100100111011111111111111
Mbita 20 11111101101111001111101011111111111111111
Mbita 21 0111000001111000010000000001001111110011111100
Mbita 22 1110110010011111000111100101111111111111111
Mbita 23 11101111101110100111100111111111111111111
Mbita 24 11111111111111100111111111111111111111111
Mbita 25 111111011111010001111111000000000001111111
Mbita 26 11101111111111001111011000000000011111111
Mbita 27 111111111111111111111111101110111111111111
Mbita 28 111111101101111001111011011111100111111
Mbita 29 1110111111111100110101010111111111111111
Mbita 30 11111111101111001111000000011101001111111
Mbita 31 11101110110111100110101011111111111000000000
Mbita 32 11001100011010001011101011111111111111111
Mbita 33 1111111100111101010110000111111111101111111
Mbita 34 11111111111111110101011111011111101111111
Mbita 35 000000000111110001111111010111111011111111
Embu 01 0110110001111111111111111111110111011111
Embu 02 01001110001111111011111111111110111111111
Embu 03 010000100001111110000101011110110111110011100
Embu 04 1100111011111111111111111110111111111
Embu 05 1111111100011111111111111111111111111111100
Embu 06 1111111110111111111111111111111110111111
Embu 07 111111110111111111111111111111111111111
Embu 08 1111111110111111111111110111111110011111111
Embu 09 111111110111111001111111111110111101111111
Embu 10 11111111101111111111111111111111111111111
Embu 11 1111111110111111111111111110111111111111
Embu 12 1111111111111111111111111011110111111111
Embu 13 11111111000000001111111111111011101111111
Embu 14 11111111011111111111111111111110111111
Embu 15 111111111111111111111111111111110111111
Embu 16 1111111100010001011111011111111111111
Embu 17 1111111100000000111111111101111111111
Embu 18 11101111111111111111111101111111111111
Embu 19 11111111111111011111111101010111111111111
Embu 20 1111111111111101111111001111111111111
Embu 21 111111111111111111111111110111101111111
Embu 22 111111111011111111111110101101111111
Embu 23 11111011011111110000101111001101111001000
Embu 24 11111110111111111111111101011111111111
Embu 25 1111111111111111111111111111111101111111
Embu 26 11111111101111111111111111110111101111111
Embu 27 11111111111111011011111110111100111111
Embu 28 111111110011111010101111111111111011111
Embu 29 11111111101111011111111001101110111111
Embu 30 111011111111111111110110110101111111111
Embu 31 11101111111111111111110101101011111111
Embu 32 11111111111111111110110111111111110
Embu 33 1110111111111111111111001111111111111
Embu 34 1111111110011111111111011111111111111
Embu 35 00000000000000000011111110111111101111111
Puerto Rico 01 11001100001000000111100001111101011010101100
Puerto Rico 02 11001100110100011111111111000000000
Puerto Rico 03 0110111010000000011010110101111011011100
Puerto Rico 04 11101110001000000111101100111111001101010110
Puerto Rico 05 00110010001101000010000000001111101111111000
Puerto Rico 06 111101100010000001111110111111011010111010
Puerto Rico 07 110010010100100001111100001111110011111010
Puerto Rico 08 001000100100000000111011010111100000111100000
Puerto Rico 09 11101000010100000011101101111101111101100
Puerto Rico 10 1111101001100100111101010111111000000000000
Puerto Rico 11 110011000111000000111101000111111110111110000
Puerto Rico 12 110011000011100000011100100010111111101111101110

III. Rapid band scoring sheet for Banana weevils from different geographic locations

```
Zanzibar 25 11111101011111100010111110111011011111111110
Zanzibar 26 11111100111110000011011011011111111111111100000
Zanzibar 27 11111111111111110011111110011111111111111100110
Zanzibar 28 11101110011111100011101101111111100111100110
Zanzibar 29 11111101111111100001110001011111111011111101111
Zanzibar 30 11111100011110011011010010011110101011111111110
Zanzibar 31 11011110010110100001010011011100111011101111110
Zanzibar 32 11110110111110111011011011111110111110111111110
Zanzibar 33 11111100111111011011110011011100101000111111110
Zanzibar 34 11111100011111100111001101110011100011111110
Zanzibar 35 11110101001111110011111010111111101100101011110
Zanzibar 36 11111100110010100111110100111111110001111111110
Zanzibar 37 11111100011110010111100110010000010000010000
Zanzibar 38 1111110001101111001011011010100101011111111110
Zanzibar 39 11111110011111110011111100011111110111111111110
Zanzibar 40 11111110111110000101100101111111111111111110
Zanzibar 41 110101100000000000010110110111111111111000000000
Zanzibar 42 1111111101111010011011000111111010010111100010
Zanzibar 43 110000000001100000010000100101011000010100000
Zanzibar 44 1100100100000000001100001000010110100111101110
Zanzibar 45 1111111001111111101101101100110110101111111110
Zanzibar 46 11111101010111100001011011011111111111111110
Zanzibar 47 11111100111110100001101101001011111101101010100
Zanzibar 48 1111010011111010001111110111111111001111111110
```

IV. RAPD band scoring sheet for Banana weevils from various locations in Uganda

OPA17-01 OPA17-02 OPA17-03 OPA17-04 OPA17-05 OPA17-06 OPA17-07 OPA17-08 OPA17-09 OPO18-01 OPO18-02 OPO18-03 OPO18-04 OPO18-05 OPO18-06
OPO18-07 OPO18-08 OPO18-09 OPO19-01 OPO19-02 OPO19-03 OPO19-04 OPO19-05 OPO19-06 OPO19-07 OPO19-08 OPO19-09 OPO19-10 OPO20-01 OPO20-02
OPO20-03 OPO20-04 OPO20-05 OPO20-06 OPO20-07 OPO20-08 OPO20-09

Ntengeru 01 1011111000101000000111011110101110110
Ntengeru 02 1111111001111111011101110011101110
Ntengeru 03 11111110111111110011101111011110
Ntengeru 04 111111101111101110110110110111111110
Ntengeru 05 1111111001111111111110110110111111010
Ntengeru 06 1111111011111111011111111111111110
Ntengeru 07 11111110111101101011111110111111110
Ntengeru 08 111111101111111110111111111111110
Ntengeru 09 1111111011111010110110110110111111110
Ntengeru 10 111111101111111101111111011111111111
Ntengeru 11 111111100111111111011111111011111110
Ntengeru 12 11111110011111111011111111010111111111
Ntengeru 13 0011011100111111110011111111011111111
Ntengeru 14 00000000010100011011110110110111111000
Ntengeru 15 11111110011111111101110110110111111110
Ntengeru 16 1111111001111111101111111111111111111
Ntengeru 17 000000000111111101101101101111111110
Ntengeru 18 1111111001111110110110110110011111110
Ntengeru 19 111111100111111111011101101111111111
Ntengeru 20 1111101011111111101111111011111111111
Ntengeru 21 111110100111111011011101101100011000000
Ntengeru 22 10011011011110000111111111111111000000
Ntengeru 23 11011110111111111110111111111111110
Ntengeru 24 11111010011011101101111111101111000010
Ntengeru 25 01111110011011100101111111111111011110
Ntengeru 26 111111101111111111011101101111011111111
Ntengeru 27 011111101111111110111101101111011111111
Ntengeru 28 11111110011111111011101101111111111110
Ntengeru 29 0000000010100000001111111011110000

Kawanda 01 01001000011111111011100101000111111100
Kawanda 02 0010111111111111010101101010001111110
Kawanda 03 01000010110111100011111101001111100000
Kawanda 04 0110111001111111011110101101101111110
Kawanda 05 0100111001111111011110111011110111111110
Kawanda 06 01111111111111111001110110111111111110
Kawanda 07 010010111111111010101101100111111100
Kawanda 08 011111111111111110011111111110111101100
Kawanda 09 1111111101111111011111111110000000000
Kawanda 10 0110111101110001010111111001111111111
Kawanda 11 0111111111110010111111111110111111111
Kawanda 12 10101110011111001011111111011111111111
Kawanda 13 1011001001111100100111111111100111111011
Kawanda 14 10101110111111010101111111100111111111
Kawanda 15 1010101001011110101101111011111011111010
Kawanda 16 11101010011111101101101101111111111110
Kawanda 17 01101110011111101111111111100111111110
Kawanda 18 01101110011111111011111111100111111111
Kawanda 19 00101100001111110111111111100111111000
Kawanda 20 11111111111111101110110101110011111010
Kawanda 21 0011111110000000001111111101111111001
Kawanda 22 000000000111100100000000000011111000
Kawanda 23 10111010011111011100111111111100111111011
Kawanda 24 00000000011111110111111111110011101011
Kawanda 25 111111010011100110011011111111000000
Kawanda 26 0111111101111011111111111110011011110
Kawanda 27 0111111011111111100000000000011000000
Kawanda 28 000010100111111110000000000000110100000
Kawanda 29 10011011000000000001100000001111111010

Namunyonyi 01 1110101101101111001111111111000000000
Namunyonyi 02 1111101011001111111011111111111110
Namunyonyi 03 11111111001001111111110111111111101110
Namunyonyi 04 1111101911011111100010000001111111110
Namunyonyi 05 101110100110111111101111111111111110
Namunyonyi 06 111110100100111101111011111111101110
Namunyonyi 07 110010101100101000111101111111111110
Namunyonyi 08 00111010001010110011101101111111111110
Namunyonyi 09 111110101010101101111111111111111110
Namunyonyi 10 10111010011011111011011011111111110
Namunyonyi 11 000000000111101000000000000111011110
Namunyonyi 12 110110111110010001100111111111011110
Namunyonyi 13 1111101011011111011111111111011100
Namunyonyi 14 110010160110111110111111111111111110
Namunyonyi 15 111110100110000111011111111111110
Namunyonyi 16 1111100000111100011101111011101110
Namunyonyi 17 11111010011111111111011111111111110
Namunyonyi 18 0000000001011110111111111111111110
Namunyonyi 19 11011011010010111111111111111011110
Namunyonyi 20 11111011010010111111111101111111111110
Namunyonyi 21 11001011011111010111111111111111110
Namunyonyi 22 01111010011100100011101101111111111110
Namunyonyi 23 01110000000000000111011101111111110
Namunyonyi 24 10000000011111011100000000001110100
Namunyonyi 25 11001011111000000011101111111011110
Namunyonyi 26 011110110111111111110111111111111110
Namunyonyi 27 1011000011101000111011111111110000
Namunyonyi 28 1111101001111101011111111111100000
Namunyonyi 29 0011010010111111111111111111000000000

Ntungamo 01 000111001000110001011111100111001100
Ntungamo 02 11111111111111101101111111101111111
Ntungamo 03 111111011111111111011111101111111
Ntungamo 04 1111111111111111011110110110111110
Ntungamo 05 101111110000100001011110101101110001111
Ntungamo 06 1111111010110111111111111101111111
Ntungamo 07 11111110111111011111111111101111111
Ntungamo 08 1111111111110011010010111111111111
Ntungamo 09 1011111111111111011111111011101111
Ntungamo 10 1111111011110101111011111101111111
Ntungamo 11 1111111011110101101101111101111111
Ntungamo 12 0111111011101010111111111111111111
Ntungamo 13 1011111011110101101111111111111111
Ntungamo 14 11111110111111011111111111111111
Ntungamo 15 01111110111011011001011111111111111
Ntungamo 16 1111111111110101111111111111111111
Ntungamo 17 11011111111010101111111111111000010
Ntungamo 18 11111110011111111111101111101111111
Ntungamo 19 00111110111110101111011111111111111
Ntungamo 20 111111111111110110000000000011101111
Ntungamo 21 111111100110111111111111111101111111
Ntungamo 22 11111110111111111111111111011111111
Ntungamo 23 0111111101101101001111111101101111
Ntungamo 24 011111111111101111101111111000000000
Ntungamo 25 10111110101110111101111111111111111
Ntungamo 26 001111110001101101010111111111111111
Ntungamo 27 10110111000010000011011101111111110
Ntungamo 28 0011111001111101001101111111111111
Ntungamo 29 000000000000000000111011111111011111

Bundagara 01 111111101101011111110101000100100
Bundagara 02 11111111111110110110110101000110111011
Bundagara 03 111111111111101101101111101011111
Bundagara 04 1111111111110111111111100111111
Bundagara 05 11111111111101111010110111011011111
Bundagara 06 0000000001111111011010110111111000
Bundagara 07 1111111011110110111011001011111100
Bundagara 08 1111111111111011011111101111110
Bundagara 09 1111111111110110111111111011111111
Bundagara 10 110111001111111111101111111111100
Bundagara 11 000000000111011111110101101011110
Bundagara 12 11111011011011111111111001111110
Bundagara 13 111111110111111011111110011111011
Bundagara 14 11010100011010101111101001110011000
Bundagara 15 1111001011000010110110001111111000
Bundagara 16 1111111011011010110110111111010
Bundagara 17 111111001111111011101111011111110
Bundagara 18 11111100000000000111011110111111110
Bundagara 19 1101010111111101111101110011011111
Bundagara 20 111111110111111101111011110011111111
Bundagara 21 1011011011011101000000000011011011
Bundagara 22 1111110011111011111101011111010
Bundagara 23 111111111111011001101110011111111
Bundagara 24 1111110011111111111110000000000
Bundagara 25 11111101011101011011111111011111
Bundagara 26 1111110011110110110111111011111111
Bundagara 27 10110110111110101101111011011110
Bundagara 28 10010110011111011010101001001011110
Bundagara 29 1111011101101101010100011001100100000

Namubiru 01 11111101111111000000000011111111
Namubiru 02 00000000011111111111011101111111
Namubiru 03 000000000111111110101110000111111
Namubiru 04 1111111011111111111111111111111
Namubiru 05 1111111011111111111111111111111
Namubiru 06 0000000000000000000111111011000000000
Namubiru 07 111111100000000000111111111111111
Namubiru 08 00000000011111111111111111111111
Namubiru 09 111110100111111101111111011111111

IV. RAPD band scoring sheet for Banana weevils from various locations in Uganda

```
Namuhiru 10 11111111111111111111111111111011111111
Namuhiru 11 11111111111111111111111111111011111111
Namuhiru 12 11111111111111111111111111111011111111
Namuhiru 13 11111111111111111111111111111011111111
Namuhiru 14 11111111111111011011111111111011111111
Namuhiru 15 11111111111111111111111111111011111111
Namuhiru 16 11111111111111111111111111111011111111
Namuhiru 17 00000000011111111111111111111011111111
Namuhiru 18 11111111101111111100000000001111111111
Namuhiru 19 11111110011111111111111111111000000000
Namuhiru 20 11111110011111111111111111111000000000
Namuhiru 21 11111111111111110111111111111011111111
Namuhiru 22 11111111100100000011111111111011111111
Namuhiru 23 10011010011111111111111111111011111111
Namuhiru 24 11111110011111101011111111111011111111
Namuhiru 25 00000000011111101000000000000011011111
Namuhiru 26 10111111001111010111111111111011111111
Namuhiru 27 00000000011111101000000000000011111111
Namuhiru 28 00000000001000000011111111111001111111
Namuhiru 29 00000000011111011111111111111000000000
Buzaya 01 10111110011111011111110111101111111110
Buzaya 02 11111110111111111111111111111011111111
Buzaya 03 11111111111111111100100011010111111110
Buzaya 04 11111111111111111111111111111110111
Buzaya 05 10111010011111111111111101111111110111
Buzaya 06 11111110011111111011110110110111111110
Buzaya 07 11111110111111110111101101111111111111
Buzaya 08 11111110111111110111101101101110111111
Buzaya 09 11111110111111111111111111011010110110
Buzaya 10 11111111111111111111110011110111011
Buzaya 11 11111111111111111111111111110101111110
Buzaya 12 10111010011110111111110111000111111011
Buzaya 13 11111010011111111111100111111110110111
Buzaya 14 10111110011111011011101101010110110111
Buzaya 15 11111111011111111111111011101011111111
Buzaya 16 10111111111111011111110111011011111110
Buzaya 17 11111111111111111111111011111011111111
Buzaya 18 11111110011111111111110111111110110010
Buzaya 19 10111110111111111111111111011010110011
Buzaya 20 10111110111111100001111111111011111111
Buzaya 21 11111110100000000001111111111110110011
Buzaya 22 11111110010010010011101111011110110100
Buzaya 23 11111111111111111111010111011011111011
Buzaya 24 11111111111111010100111111111011110100
Buzaya 25 10011010011110111111111111111010111011
Buzaya 26 11111111011111101011111101111010111111
Buzaya 27 11111110111111111011101011111110000000
Buzaya 28 11111110011111110110111010101101111111
Buzaya 29 11111111111111111111111011111000000000
Kabukye 01 10101001011111011111110000011100000
Kabukye 02 10111011011110011001010111001010111110
Kabukye 03 11111111011111111011110111011111111110
Kabukye 04 11111011010111101011011010000111111111
Kabukye 05 11111111011111100011100010100111111111
Kabukye 06 01111100011011101011111111011111111111
Kabukye 07 11111101011011011111111111010111111110
Kabukye 08 11111001011111011111101011110111111010
Kabukye 09 11111111011111101000000110111111011
Kabukye 10 11111110011111011111101111011111111111
Kabukye 11 10110000011110011111101010101110110111
Kabukye 12 11111101011111111011111111011111111110
Kabukye 13 11111110111111011111011111111110111
Kabukye 14 11111111011111111011011111011111110
Kabukye 15 11111101011111011111101010111111110
Kabukye 16 11111111011111000111111010101111111111
Kabukye 17 11111111011111011011011010101111111
Kabukye 18 10110000001111010111111010111111111
Kabukye 19 11111011011111011111111111011111111
Kabukye 20 11001010001111011111100110011110111
Kabukye 21 11100001011111101000000000011010000
Kabukye 22 11100011011111011111101001000111101110
Kabukye 23 10100001011111111101100111111110111
Kabukye 24 10110000011111011111101100011110100
Kabukye 25 11111110101000000110001100000010000
Kabukye 26 11000000011110110111100000001001110
Kabukye 27 11111110000000001101111111111100
Kabukye 28 11111001011101101011110110011111111
Kabukye 29 11111110111111111111111111011111111
Kalegweri 01 11111110100100000111010100111110110
Kalegweri 02 001111100100000011111110001111111
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Kalegweri 03 01111111111111011111111011111111111
Kalegweri 04 00001000011111011111111110000000000
Kalegweri 05 11111100111111011111111111111111111
Kalegweri 06 11111111100000000111111111101111111
Kalegweri 07 11111111111111011111111111111111110
Kalegweri 08 01111110111111011111111111011111111
Kalegweri 09 11111100101100110111111100111111111
Kalegweri 10 11111111111111011111111011011011111
Kalegweri 11 11111111111111011111111110011011111
Kalegweri 12 11111111111111010111111111011011111
Kalegweri 13 00111010011111110000000000110111111
Kalegweri 14 11111111111111010111111110011111111
Kalegweri 15 11111111111111111111111111110111111
Kalegweri 16 11111101111111010111111111111011110
Kalegweri 17 00000000111111011111011111101111111
Kalegweri 18 11111110000000000111111110111111111
Kalegweri 19 00000000010000000011111111011111110
Kalegweri 20 11111111111111010111111110011011110
Buwama 01 10110110001110010111111110001101111
Buwama 02 11110100011110010111111110010111111
Buwama 03 11110110011110010111111110111111111
Buwama 04 11110110001111010111111111011111111
Buwama 05 11111110001111110111111110000000000
Buwama 06 10111110001111001011110100111101111
Buwama 07 10111110111111010111111111011111111
Buwama 08 10111110001111010111111110000000000
Buwama 09 11111110011110010111111110011111111
Buwama 10 11111110011111001011111110011111111
Buwama 11 11111110001111010111111110010111111
Buwama 12 11111110011110101111101100000101110
Buwama 13 11111110011111010111110110011011111
Buwama 14 11111000001111010111111011001110111
Buwama 15 11111110001111110111111101111011011
Buwama 16 10111110011111101111111110000101111
Buwama 17 11111110011111110111111100011111111
Buwama 18 11111110001111010111110110010011111
Buwama 19 10111110011111110111100110000000000
Buwama 20 11111101111111011111111111001011111
Buwama 21 11111101111111011110011001001011
Buwama 22 11110100011111101111010100000000000
Buwama 23 11111110011100010111111110000000000
Buwama 24 11111110111111011111011111000000000
Buwama 25 11111101111111011100110000000000
Buwama 26 11110100011111110111111110011111111
Buwama 27 11111110001100000111111110111111111
Buwama 28 11111100111011101111011110111111100000
Buwama 29 11111111111011110000000000000000000
Bukanga 01 10111100111111100000000000110111111
Bukanga 02 11111100111111011111111111101011
Bukanga 03 000111101111111111111111011111111001
Bukanga 04 11111111111111111111111101111111111
Bukanga 05 11111100100100100101111110111101011
Bukanga 06 00001100100110101111111011111001
Bukanga 07 11111110111100100000000000011111001
Bukanga 08 11111101111111101111011111011111
Bukanga 09 00111100111111111111111111011011
Bukanga 10 00111100100000101111111111011101
Bukanga 11 11111101111111011111111111111001
Bukanga 12 11111111111111111111111011110001000
Bukanga 13 00000100011101111111111111111011011
Bukanga 14 10111110111111111111111111111111
Bukanga 15 11101111011111010111111111111001
Bukanga 16 10011010011101111110011111011011
Bukanga 17 10111111111111011111011111111111
Bukanga 18 10111110111111111111111111111001
Bukanga 19 11111111111111111111111111111111
Bukanga 20 00000000010110111001110101111101
Bukanga 21 10111111111111011011111111111111
Bukanga 22 10111111000000001111111111111111
Bukanga 23 11111110111101000111111011111001
Bukanga 24 11111110111111011011111111011111001
Bukanga 25 10111110111100010011101011111111
Bukanga 26 11111110111111111111111111111111
Bukanga 27 10111111011111011011111011111111
Bukanga 28 10111111111111011111111000100000000
Bukanga 29 010111110100001000011111111111111
Bunyaruguru 01 10110110101011100000110000100000
Bunyaruguru 02 10110010001011110001011001011100000
Bunyaruguru 03 111111101111111011110101011111111
Bunyaruguru 04 11111111101111110011010100110100
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IV. RAPD band scoring sheet for Banana weevils from various locations in Uganda

Bunyaruguru 05 00000000(0)1111111010001111111111111110
Bunyaruguru 06 1101110101100011110010011111011110000
Bunyaruguru 07 1101111101011111111010111111001110010
Bunyaruguru 08 111111110110111110000000000000011111110
Bunyaruguru 09 111111110111111110010111011011111110
Bunyaruguru 10 000010000111101100000000000001000000
Bunyaruguru 11 111111100111111111000000000011011111
Bunyaruguru 12 11111111011110111101111111000011100
Bunyaruguru 13 1111111101111111100000111001101111100
Bunyaruguru 14 10000000010101110101101001001111000
Bunyaruguru 15 11111111111111010111111111011111111111
Bunyaruguru 16 101111110101101111010001110010100
Bunyaruguru 17 111111100111110110100111111110111111111
Bunyaruguru 18 11111110211111011010011110111011111110
Bunyaruguru 19 111111110101101101101111111001010110
Bunyaruguru 20 000000000010001101111111110011100010
Bunyaruguru 21 111101110111011110111111111111111110
Bunyaruguru 22 0111011110111011100011111111111111110
Bunyaruguru 23 11111110011111111001111111001011110
Bunyaruguru 24 11111101110111111010111111110111111110
Bunyaruguru 25 1111101001110101110111111111111110010
Bunyaruguru 26 11111111111111110000000000000111001010
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