Biosystematic studies of the gramineous stemborers, *Chilo partellus* (Swinhoe) and *Chilo orichalcociliellus* (Strand) (Lepidoptera:Pyralidae).

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DECLARATIONS

This thesis is my work and has not been presented for a degree in any other university.

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In remembrance of my late grandmother, Mary Mbatha Kioko (WATENE), who died during the course of this study. Her spirit of hard work, determination and dedication has been a source of inspiration to many of us.

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ABSTRACT

On the Kenya coast, the indigenous coastal stemborer, *Chilo orichalcociliellus* (Strand), and the exotic spotted stemborer *Chilo partellus* (Swinhoe), are pests of cereals. The two species occur together occupying similar ecological niches. Earlier studies have shown that their larvae are difficult to distinguish and that the exotic *C. partellus* may be gradually displacing the indigenous species. During this study, comparative larval development investigations in the laboratory suggested that the shorter generation time could explain the superior competitiveness of the exotic *C. partellus* over the indigenous *C. orichalcociliellus*.

A study of morphological characters showed that the structure of spiracles, mandibular structures, the arrangement of setae and number of crochets on the abdominal and caudal prolegs could not be reliably used for distinguishing the two species. Asetose tubercles were shown to be useful characters in separating larvae of the two species. However, the use of asetose tubercles was limited since they were not present in all larval stages. The character therefore became unreliable for species separation.

Hexane soluble cuticular components were studied using gas chromatography. Chromatograms of the components did not show any qualitative differences in the peak patterns, nevertheless, quantitative differences were observed. Analysis of the quantitative differences suggested that the cuticular components of these species were highly influenced by the diet and/or the environment, and as such, were not a reliable character for their identification.

Electrophoretic banding patterns to three enzyme systems were found to be diagnostic. Glucose phosphate isomerase (GPI), Lactate dehydrogenase (LDH) and Glutamate oxaloacetate transaminase (GOT) on thin starch gel electrophoresis gave distinct separation between the larvae of the two species. These results showed that starch gel electrophoresis could be used as a reliable alternative technique for distinguishing larvae of these two stemborer species, especially in cases where morphological characters cannot be used.

CHAPTER ONE

1 General Introduction

The coastal stemborer, *Chilo orichalcociliellus* (Strand), and the spotted stemborer, *Chilo partellus* (Swinhoe) are lepidopteran pests of cereals. They are members of the family Pyralidae, subfamily Crambinae. *Chilo orichalcociliellus* has been recorded from Kenya, Tanzania, Zaire, South Africa, Madagascar, Malawi and Nigeria (Bleszynski, 1970; Delobel, 1975a&b; Medler 1980). However, Mohyuddin and Greathead (1970) suggested that *C. orichalcociliellus* may have been confused with *Chilo thyrsis* Bleszynski in some earlier records. The reference from Nigeria (Medler, 1980) needs to be verified as several recent surveys of stemborers in Nigeria and neighbouring countries have not reported the occurrence of *C. orichalcociliellus* (Schulthess *et al.*, 1991; Bosque-Perez and Mareck, 1990; Moyal and Tran, 1992; Girling, 1980; Macfarlane, 1990).

The distribution of *C. partellus* includes Afghanistan, Bangladesh, Cambodia, India, Indonesia, Laos, Nepal, Pakistan, Sri Lanka, Thailand and Vietnam (Bleszynski, 1970; Neupane *et al.*,

1985; Harris, 1990) and this species is probably indigenous to Asia (Mohyuddin and Greathead, 1970; Harris 1990). It was accidentally introduced to Africa earlier this century and was first reported in Malawi in 1932 (Tams, 1932). The African distribution of *C. partellus* now includes Ethiopia, Kenya, Malawi, Mozambique, Somalia, South Africa, Sudan, Tanzania, Uganda, Botswana, Swaziland, Zimbabwe, Burundi, Comoros, Djibouti, Rwanda, Cameroon and Togo (IAPSC, 1985; Harris, 1990; Sithole, 1990; Minja, 1990). However, as with *C. orichalcociliellus*, the West African reports are questionable since *C. partellus* has not been recorded in recent extensive surveys in West Africa (Schulthess *et al.,* 1991; Bosque-Perez and Mareck, 1990; Moyal and Tran, 1992; Girling, 1980; Macfarlane, 1990).

Lepidopteran stemborers are considered to be among the most damaging pests of cereal crops worldwide (Harris, 1962; Ingram, 1958; Jepson, 1954; Seshu Reddy, 1983). Plant injury is caused by larvae feeding in the plant's whorl and later through stem tunnelling. Affected plants have poor growth, reduced yield and are more susceptible to wind damage and secondary infections. Crop losses due to stemborers can be very serious particularly in early stages of growth where heavy infestations

result in 'dead heart' and stunted growth. *Chilo* species infestations can be as high as 87 % (Seshu Reddy, 1983). Grain yield losses ranging from 23-53 % in maize (Wheatley, 1961), and 2-88 % in sorghum have been recorded (Seshu Reddy, 1988).

The life cycles of *C. partellus* and *C. orichalcociliellus* are very similar. The adults are nocturnal and live for 2-3 days during which each female lays eggs in batches of 10-80 eggs on the under surface of leaves (Scheltes, 1978; Harris, 1990; Minja, 1990).The fecundity of the two species is approximately equal, with *C. partellus* ovipositing a mean of 434 eggs (Berger,1989) and *C. orichalcociliellus* a mean of 475 eggs (Delobel, 1975b).The egg development period for *C. partellus* is 4-5 days at 26-28^0 C (Berger, 1989) and 5-6 days for *C. orichalcociliellus* at 28^0 C (Delobel, 1975b). After hatching, larvae initially feed on the leaves. Older larvae bore into the main stem and larval development is completed in 2-4 weeks. Fully grown larvae prepare for pupation by cutting exit holes in the stem to enable moth emergence. The pupal period lasts 4-8 days for *C. partellus* and 6-9 days for *C. orichalcociliellus* (Delobel, 1975b). The life cycle is completed in 25-50 days in *C. partellus* (Ingram, 1958;

Neupane *et al.*, 1985; Harris, 1990) and 38-57 days in *C. orichalcociliellus* (Delobel, 1975b).

Insecticide effectiveness against gramineous stemborers is hindered by the feeding behaviour of the larvae. Applications are only effective during the limited period before the stemborers enter the protected environment of the stems (Ingram, 1983; Scheltes, 1978; Warui and Kuria, 1983). Furthermore, chemical control of stemborers is often beyond the economic reach of the resource poor farmers of the developing countries. In addition, insecticides are environmentally unsafe. More recently, research has been directed towards the development of integrated pest management (IPM) approaches to the stemborer problem. In any rationally planned attempt to manage insect pests, accurate identification of the target organism is essential (Cock, 1986; Schulten, 1987; Goto, 1982). Resistant varieties, biocontrol agents, pheromones and some insecticides used in IPM may be highly species specific and only succeed if the identity of the pest is accurately known.

Schulten (1987) stressed the continuing need for biosystematic services in the identification of insect pests.

Success in many programmes has been significantly delayed by misidentification of the pest or the natural enemies (Cock, 1986). An example of this is the Kenyan coffee mealybug, *Planococcus kenyae* (Le Pelley) (Homoptera:Pseudococcidae). The pest was first reported to be *Pseudococcus lilacinus* Cockerell. Time was wasted in exploration and introduction of the wrong natural enemies from countries such as S. Africa, California, Honolulu and Japan. Later, the pest was correctly identified and was found to have been from Uganda. A parasitic wasp of the genus *Anagyrus* Howard (Hymenoptera:Encyrtidae) was subsequently introduced from Uganda which brought the mealybug under control (Hill, 1983).

The distinction between *C. partellus* and *C. orichalcociliellus* is precise when genital morphological characters of the adult are used (Delobel, 1975a). Wheatley (1961) reported that larvae of the two species cannot be separated. He described *C. orichalcociliellus* as being similar to *C. partellus* in all aspects and could only distinguish them at the adult stage. La Croix (1967) working on maize at the Kenya coast could not distinguish the larvae of these two species. Mathez (1972) studied the pattern of asetose tubercles on the integument which were found to be

distinctive between these two species. Delobel (1975a) observed the same characters as Mathez (1972) but he cautioned on the unreliability of these larval external morphological characters. Mathez (1972) observed that some larvae of these two species enter aestivation during which time all pigmentation is lost and the tubercles disappear leaving a whitish larva. Moreover, he observed that the distinctive tubercles are absent on very young first instar larvae. Delobel (1975b) noted that loss of pigmentation and tubercles also occurred in larvae at the pre-pupal stage. The phenomena of aestivation in the two *Chilo* species has since been reported both in laboratory colonies (Ampofo, 1988; Delobel, 1975a&b) and in the field (Scheltes, 1978). Since the distinctive asetose tubercles disappear during aestivation, it becomes impossible to distinguish aestivating larvae of *C. partellus* and *C. orichalcociliellus*.

First instar larvae cannot be identified using the tubercles since in most *C. orichalcociliellus* these tubercles do not appear until 24-48 hours after hatching. In *C.partellus*, the asetose tubercles on the meso and metathorax, lateral view, appear on some larvae after the second ecdysis. The asetose tubercle posterior to the abdominal spiracles appears after the third ecdysis

(Mathez, 1972). To date, no external morphological character has been found to separate larvae of these two species during the early first instar stage (first 24-48 hours) or when larvae are aestivating (Mathez,1972; Overholt *et al.*,1993). A number of workers have used external morphological characters to distinguish *C. partellus* from lepidopteran stemborers other than *C. orichalcociliellus* (Gupta, 1940; Isaac & Rao, 1941; Trehan & Butani, 1948). They relied on setae, arrangement of crochets on prolegs, size of the body, colour, stripes and tubercles on the body, mandibles and colour and shape of spiracles.

There is need for a systematic study, with particular emphasis on the identification of reliable characters for the distinction of the larval stages of these two species. In this study morphological characters that are likely to be consistent in all the larval stages have been examined. Biochemical characters have also been investigated. Availability of reliable distinctive characters will be of great value to researchers conducting population studies on stemborers. Information on the development of the pest is important for the development of IPM programmes since the length of time an insect spends during the vulnerable life stages may affect rates of attack by natural enemies, thereby influencing

the population dynamics of the insect. Comparison of development data of *C. partellus* and *C. orichalcociliellus* larvae may also provide insight into biological differences which may be influencing their relative abundance at the Kenya coast.

1:2 Main Objective:

The general objective of this work is to identify reliable taxonomic characters that can be used for separation of *C. partellus* and *C.orichalcociliellus* larvae and also to study the larval development of the two species under controlled laboratory conditions.

Specific Objectives:

1. Determine and compare larval development period and number of instars for *C. partellus* and *C. orichalcociliellus* under laboratory conditions.

2. Evaluate external morphological characters for separation of larvae of *C. partellus* and *C. orichalcociliellus*.

3. Analyse hexane extracts of cuticular components using gas chromatography to determine if their composition is of reliable taxonomic use for distinguishing these two borer species.

4. Analyse enzyme systems using thin starch gel electrophoresis and evaluate the suitability of enzyme systems for separating *C. partellus* and *C. orichalcociliellus* larvae.

CHAPTER TWO

2 Literature Review

2.1 Taxonomy of the genus *Chilo*

The genus *Chilo* was erected by Zincken in 1817 (Zincken,1817). It is a large genus including 41 species. All *Chilo* species are stemborers of gramineous plants and some are recognized as serious pests of cereal crops (Bleszynski, 1970). Bleszynski (1970) gave a revision of the taxonomy and the geographical distribution of the 41 species of *Chilo* with brief discussions on their biology and economic importance. Most of the *Chilo* species are represented in the Ethiopian and Oriental regions with 17 species occurring in Africa. Only one species, *C.chiriquitensis* (Zeller), is confined to the Neotropical region, while four are confined to the Nearctic region. Taxonomy of larvae in this genus is based solely on morphological characters such as colour, size, position and density of setae and tubercles (chitinized portions on the body) (Nye,1960; Bleszynski,1970; Mathez,1972). It has proved to be extremely difficult to find stable characters on which to separate larvae since the

characters have been found to vary even within individuals of the same species at different ages (Gupta, 1940; Nye, 1960; Mathez, 1972; Scheltes,1978; Delobel, 1975a&b).

2:2 Abundance of *Chilo partellus* and *Chilo orichalcociliellus* at the Kenya coast

In Kenya, the introduced *C. partellus* and the indigenous *C. orichalcociliellus* are injurious to maize and sorghum (Mathez, 1972; Warui and Kuria, 1983; Scheltes, 1978). *Chilo orichalcociliellus* is confined to the coastal belt along the Indian ocean while *C. partellus* is widely distributed in the same region and in other areas below 1700 m above sea level (Minja, 1990; Nye, 1960). Mathez (1972) who conducted research work at the Kenya coast from 1965-1969 reported that *C. orichalcociliellus* was the predominant stemborer species in maize. Later, Warui and Kuria (1983) reported that *C. orichalcociliellus* was predominant during the long rains and *C. partellus* during the short rains. Recent studies at the Kenya Coast have shown that *C. partellus* was by far the most abundant species during the long and short rainy seasons in 1991 and 1992 (Overholt *et al.*, 1993). This historical shift in species abundance suggests that the introduced

C. partellus may be gradually displacing the indigenous *C. orichalcociliellus*.

2.3 Taxonomic context of *Chilo partellus* and *Chilo orichalcociliellus* larvae.

Chilo partellus and *C. orichalcociliellus* are ecologically similar species and their distinction is quite delicate (Delobel, 1975). Nye (1960) described the larvae of *C. orichalcociliellus* as similar to those of *C. partellus* and found it impossible to separate them in mixed batches obtained from the field. Although he also found the adults to be superficially similar, *C. orichalcociliellus* could be identified by its gold fringed wings. Wheatley (1961) and La Croix (1967) were also unable to separate larvae of these two species and described them as morphologically similar.

Mathez (1972) was the first worker to develop a method of distinguishing larvae of *C. orichalcociliellus* and those of *C. partellus*. He reported that the best criterion was the presence of an anterior asetose tubercle on the dorsal sides of the meso- and metathorax on *C.orichalcociliellus* larvae which was absent on *C. partellus* larvae. Mathez also observed that in *C. partellus* larvae

an asetose tubercle was present posterior to each spiracle on the lateral sides of the first to seventh abdominal segments. In *C. orichalcociliellus* larvae, these asetose tubercles were absent. Additionally, on *C. partellus* asetose tubercles were present on the lateral sides of meso- and metathorax, but were absent in *C. orichalcociliellus* larvae.

The distinguishing characters mentioned by Mathez (1972) were not always valid because larval pigmentation is absent during certain growth stages. For example, it has been observed that some do not pupate at the expected time, but instead enter an aestivation period. The body colour and tubercles gradually disappear in aestivating larvae making their identification impossible (Mathez, 1972; Scheltes, 1978; Delobel, 1975a&b). The distinctive tubercles were also absent on very young first instar larvae (Mathez, 1972), and loss of pigmentation and tubercles at the pre-pupal stage was reported in *C. orichalcociliellus* (Delobel,1975a).

2.4 Biochemical systematics

Systematists have traditionally relied primarily on morphological criteria to distinguish taxa, but many groups have defied satisfactory definition (Boileau and Hebert, 1988). In such cases, biochemical techniques may have application. Since the separation of *C. partellus* and *C. orichalcociliellus* on morphological basis is unsatisfactory, studies to identify biochemical characters may be useful. Two of the techniques used in biochemical systematics are cuticular component analysis and electrophoretic studies of enzymes. This is the first time biochemical techniques are being used for systematic studies of these two species. From the success demonstrated in other studies using biochemical techniques (see below), it is hoped that new characters will be identified that can be used to accurately separate larvae of *C. partellus* and *C. orichalcociliellus*.

2.4.1 Cuticular component analysis

The cuticular components of arthropods play a major role in the regulation of water loss and also protect them from invasion by micro-organisms and xenobiotic chemicals (Hadley and

Jackson, 1977; Hadley, 1977; Renobales and Blomquist, 1983). In some species, cuticular components have been shown to be involved in intraspecific communication (Howard and Blomquist, 1982; Nelson *et al.*, 1981).

Cuticular component analysis has been shown to be of important taxonomic value by some workers (Carlson and Service, 1980; Hassane, 1990; Daly and Gregg, 1985; Kaib *et al.*,1991). Several authors have shown that cuticular components consist mainly of hydrocarbons with varying amounts of alcohols, aldehydes, fatty acids, esters and triglycerides (Dwyer *et al.*,1986; Renobales and Blomquist, 1983; Howard and Blomquist, 1982; Lok *et al.*, 1975; Jackson, 1981). Cuticular components have been used as a tool to differentiate morphologically similar insect species. This is because the main classes and composition of cuticular components may be considered a product of an insect's genotype, and thus available for taxonomic use (Lockey, 1991). Kaib *et al.* (1991), demonstrated that profiles of cuticular hydrocarbons are a valuable tool, providing characters for analysing complex systematics of some termite groups. They noted that hydrocarbons can be used as a tool in taxonomic studies without requiring detailed knowledge of their chemical

composition. They assumed that cuticular hydrocarbons are stable metabolic end products of a genetically controlled synthesis. The technique is potentially useful for field workers because most cuticular components are chemically stable. Thus, one can utilise live or even old pinned specimens (Carlson and Service, 1980; Kaib *et al.*, 1991).

Separation of morphologically similar taxa in insect groups by cuticular components has been used. For example, Carlson and Service (1980) were able to identify mosquitoes of the *Anopheles gambiae* Giles (Diptera: Culicidae) species complex through the analysis of their cuticular components. The two important vectors of malaria in Africa, *A. gambiae* Giles and *A. arabiensis* Patton often occur sympatrically and cannot be distinguished morphologically. Statistically significant differences were seen between species when selected pairs of peaks were compared. Similarly, vectors of river blindness in West Africa, female blackflies of the *Simulium damnosum* Theobald (Diptera: Simuliidae) species complex (*S. sirbanum* Vajime and Dunbar and *S. squamosum* (Enderlein)) have been separated by the analysis of their cuticular components (Carlson and Walsh, 1981). These authors found statistically significant differences between the two

species for each of the five sets of consistently appearing peaks that were quantified and compared. Hassane (1990) compared the cuticular component patterns of sandflies in Kenya and observed qualitative differences among eight species. He reported that multivariate analysis of the species *Phlebotomus elgonensis* Ngoka, Madel and Mutinga and *P. pedifer* Lewis, Mutinga and Ashford (Diptera: Psychodidae), which are morphologically very difficult to differentiate, showed significant differences. Similar success was achieved by Lockey (1978) who was able to separate two closely related tenebrionid beetle species by the analysis of their cuticular hydrocarbons. In addition Lok *et al.* (1975) reported that the cuticular lipids of the imported fire ants, *Solenopsis invicta* Buren and *S. richteri* Forel (Hymenoptera: Formicidae), had distinguishable hydrocarbon patterns.

Despite the successes demonstrated above, some workers have reported findings which imply that cuticular components are not suitable for taxonomic use in some insect groups. Jackson (1981) reported on the characterization of the surface lipids of three acridids belonging to the genus *Melanoplus* Stal (Orthoptera: Acrididae). The surface lipids of these grasshoppers showed some quantitative differences but qualitatively they had similar

chromatograms. These chromatograms were also similar to other *Melanoplus* species previously studied. Similarly, Lockey (1978) reported that congeneric species are likely to have qualitatively similar hydrocarbon mixtures. He observed that 70% of the cuticular hydrocarbons secreted by *Tenebrio molitor* (L.) and *T. obscurus* (F.) (Coleoptera: Tenebrionidae) were identical. Diet-dependent differences in cuticular components have also been reported. Baker *et al.* (1978) reported that addition of chemicals to the diet alters the surface chemistry of the insect, while Blomquist and Jackson (1973) reported that the grasshopper *Melanoplus sanguinipes* (Fabricius) can incorporate dietary hydrocarbons into cuticular lipids. A correlation between the chemical composition of the lipids from an insect's cuticle and that of the lipids from the cuticle of its host plant was reported by Richter and Krain (1980).

In the order Lepidoptera, cuticular component analysis work includes that of Bell *et al.* (1975), Coudron and Nelson (1978), Renobales and Blomquist (1983), Dwyer *et al.* (1986) and Espelie and Brown (1990). Most of this work has not been for taxonomic purposes, but it does have implications for the taxonomic use of cuticular component analysis. Renobales and

Blomquist (1983) found that the hexane extractable cuticular lipids of the cabbage looper, *Trichoplusia ni* Hubner (Lepidoptera:Noctuidae), was comprised mostly of hydrocarbons and the types of hydrocarbons varied with developmental stages. These workers also noted that although cuticular components of many insects had been examined, little attention had been given to the order Lepidoptera. Dwyer *et al.* (1986) also studied *T. ni* and reported that the shed skin had the same amount of cuticular hydrocarbons as the previous stadium. This suggests that this insect species does not re-absorb and re-utilise cuticular hydrocarbons.

Bell *et al.* (1975) observed a difference in the amount and rate of production of cuticular wax in diapausing and non-diapausing pupae of the tobacco hornworm, *Manduca sexta* Linnaeus (Lepidoptera: Sphingidae). Diapausing pupae had a faster rate of production of cuticular surface lipids than non-diapausing pupae. When the amount of surface lipid remained constant (2 weeks post pupation), the diapausing pupae had three times more surface lipid than the non-diapausing pupae. In contrast, Coudron and Nelson (1978) found no difference in the amount of cuticular surface lipid on diapausing pupae and on non-diapausing pupae of

tobacco budworms, *Heliothis viriscens* Fletcher (Lepidoptera: Noctuidae). Additionally, they did not find any differences in the amount of cuticular surface lipids on male and female pupae in both diapausing and non-diapausing stages of the development. These studies also showed that the percentage composition of n-alkanes found in the diet (which resulted from paraffin wax used to harden the food base material) was different from that found in the surface lipid of the pupae. Hence, the insect may have degraded the n-alkanes consumed in the diet and synthesized it's own cuticular n-alkanes, selectively altered the composition of n-alkanes in the diet, or excreted them and synthesized its own cuticular n-alkanes from some other carbon source.

Diet-related differences in cuticular lipids have also been reported in the order Lepidoptera. Espelie and Bernays (1989) examined fourth instar larvae of *M. sexta* reared on artificial diet or growing plants and found significant differences in the proportion of the cuticular components. They also observed a relationship between the insect cuticular lipid components and the surface components of the host plant. Cuticular lipids of the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), have been studied by Espelie and Brown (1990). They did not find any

detectable differences between the surface lipids of males and females of either larvae or adults. Apple, *Malus pumila* (Mill.) (Family: Rosaceae), is the main host plant of this moth. The major cuticular hydrocarbons of the codling moth larvae were identical to those extracted from the cuticle of the fruit and seed coat of apple. Their results demonstrate that there is a strong similarity in the surface chemistry of species which interact on tropic levels.

2:4:2 Electrophoretic variation of enzymes

Electrophoretic techniques have often been used with success in classifying taxonomic relationships of a wide variety of plant and animal species complexes (Britton-Davidan and Amoureux, 1982). Gel electrophoresis is by far the most widely used biochemical technique in insect systematics. Proteins have a charge and are therefore able to move when placed in an electric field. Different proteins have different charge and therefore will migrate at different rates. The proteins used in systematics are usually enzymes. The catalytic activity of an enzyme can be used to mark its position on the gel using enzyme-specific substrates and stains. Harris and Hopkinson (1978) described the general

techniques employed with the different support matrices for electrophoretic separation of enzymes.

In recent years, electrophoretic separation of multiple molecular forms of enzymes (isoenzymes) has been applied to clarify the taxonomic status of pest species complexes (Miles,1978). The successful use of electrophoresis to analyse enzyme patterns for the identification of species or populations within morphologically uniform groups of insects has been demonstrated by many workers. Kreutzer and Galido (1980) used isoenzyme patterns from cellulose acetate to study two species of mosquitoes. They successfully differentiated the larvae, pupae and adults by their distinct banding patterns for the enzymes acid phosphatase, glyceraldehyde-3-phosphatase dehydrogenase, phosphogluco isomarase and zanthine dehydrogenase. Other isoenzymes examined could not be used for specific identification due to similarities in their migrations. Blackman and Spence (1992) compared electrophoretic mobility of the enzyme glutamate oxaloacetate transaminase on cellulose acetate among two species of aphids, *Myzus persicae* (Sulzer), and *M. nicotianae* (Blackman) (Homoptera: Aphididae). The morphological differences between the two were very slight and could only be

demonstrated reliably by canonical variate analysis involving 11-14 characters. Electrophoresis provided a means of distinguishing individual specimens of the two species. Similar success has been reported in *Drosophila* Fallen (Diptera: Drosophilidae) (Ayala *et al.*, 1972) and in sandflies (Rogo *et al.*, 1988; Hassane, 1990).

In Lepidoptera, Pashley (1986) conducted electrophoretic surveys of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae). Thirty-five enzymes were examined and five of these were shown to be diagnostic for the various host strains of *S. frugiperda*. Pashley *et al.* (1990) also investigated the sugarcane borer, *Diatraea saccharalis* (F.) (Lepidoptera:Pyralidae). Fourteen loci were examined and significant differences were observed between Brazilian and Louisiana-Mexican populations at all polymorphic loci and at one diagnostic locus. Daly and Gregg (1985) used cellulose acetate to examine enzymatic variation in two Australian species of *Heliothis* Ochsenheimer, *H. armigera* (Hubner) and *H. punctigera* Wallengren. Two enzymes clearly distinguished these two species. In the same genus, Sluss *et al.* (1978) electrophoretically compared a field population of *H. viriscens* (F.) with both field and laboratory populations of *H. zea* (Boddie) with respect to genetic variation at 19 enzyme loci. They found

tetrazolium oxidase-A and tetrazolium oxidase-B to be diagnostic loci with complete discrimination between the two species. Esterase isoenzymes of the diamond back moth, *Plutella xylostella* L. (Lepidoptera : Yponomeutidae), was investigated by Murai (1990). He reported at least six alleles on the Est-1 locus. These alleles were useful in analysing the different gene frequencies in four populations. Harrison and Vawter (1977) used electrophoretic separation to differentiate between two pheromone strains of the European corn borer, *Ostrinia nubilalis* (Hubner) (Lepidoptera: Pyralidae). Analysis of allele frequency differences did not reveal whether the two strains should be considered distinct species.

Electrophoresis on diapausing Lepidoptera larvae includes the work of Sluss *et al.* (1975). Diapausing and non-diapausing pink bollworm larvae, *Pectinophora gossypiella* (Saund.) (Lepidoptera:Gelechiidae), were assayed for 15 enzymes. Two respiratory enzymes, lactic acid dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase, showed changes in mobility between diapause and non-diapause larvae. By late diapause, there was a loss of activity for both enzymes. Abd El Fattah *et al.* (1972) studied some enzymes in the same species,

P. gossypiella, during induction and termination of diapause. The four enzymes assayed were found to change in mobility during diapause.

CHAPTER THREE

3 General Materials And Methods

3:1 Insect collection site

Stemborers were collected from maize and sorghum at seven sites (Jilore, Dida, Kaloleni, Kilifi, Mtwapa, Patanani and Kikoneni) along the coastal strip of Kenya (Fig.1). Two of these sites are government agricultural research institutions (Kenya Agricultural Research Institute - Mtwapa station and Kilifi Institute of Agriculture). The other five sites are farmers fields which had been selected by the ICIPE/WAU project research team. This area was chosen because the two species occurred together thus permitting a comparative study. The coast also has a history of high populations of stemborers (Nye, 1960; Wheatley, 1961; La Croix, 1967; Mathez, 1972; Scheltes, 1978; Warui and Kuria, 1983; Overholt *et al.*, 1993). The collections were made during rainy seasons (December, 1992; July, 1993) and dry seasons (September, 1992; March, 1993). Maize and sorghum plants were selected based on visible plant damage. The plants were then dissected, larvae removed, placed in jars and provided with stems for feeding.

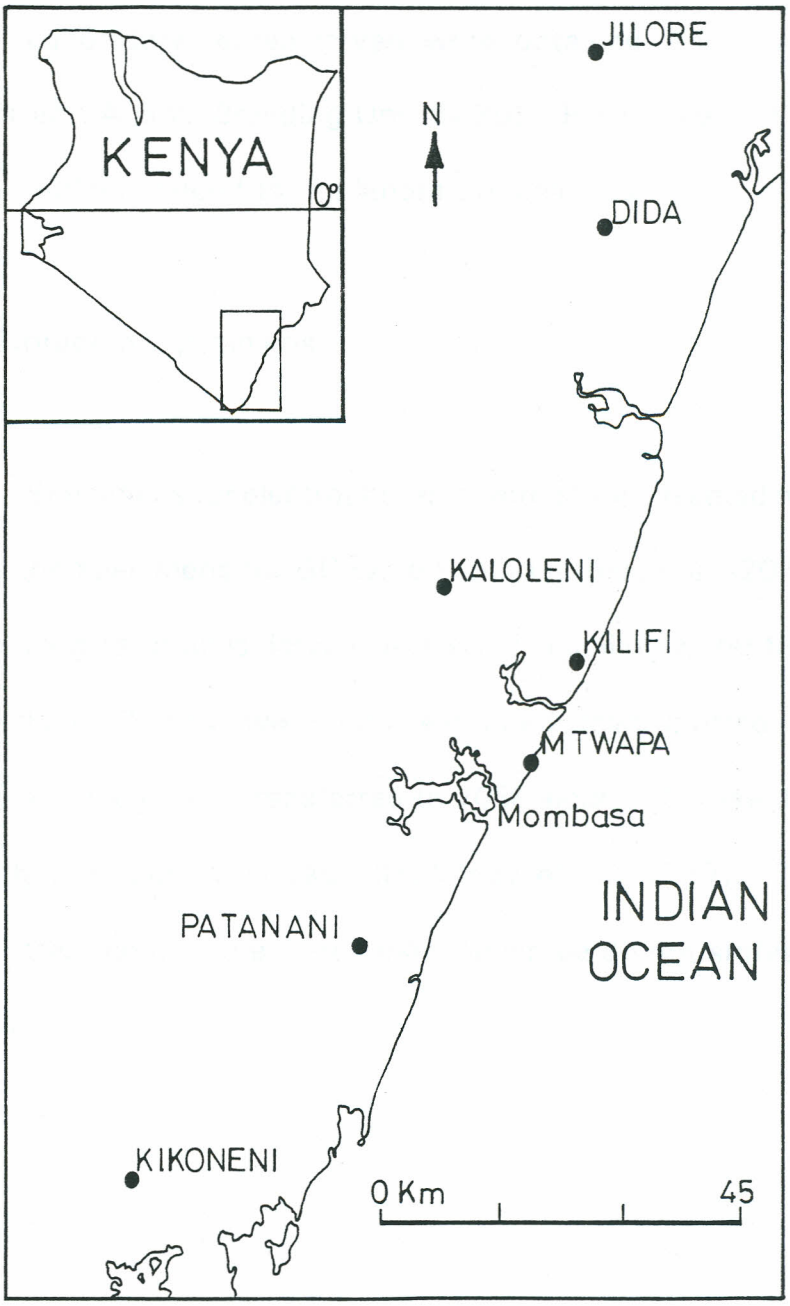

Fig.1: Map of Kenya showing sites where samples of *Chilo partellus* and *C. orichalcociliellus* were collected.

3:2 Laboratory reared larvae

Laboratory reared larvae were obtained from the ICIPE Insect and Animal Breeding Unit (IABU). Field collected insects were routinely added to the laboratory colonies.

3:3 Storage of specimens

Specimens for electrophoresis were stored in liquid nitrogen while the specimens for GC were kept in a freezer at -20° C. For morphological studies, larvae were killed in boiling water for a few seconds until they were fully extended then blotted dry on absorbent paper and transferred to 70% alcohol. Larvae dropped directly into alcohol blacken (Holloway *et al.*, 1987). The hot water treatment also ensures that the larvae do not shrivel (Nye, 1960).

CHAPTER FOUR

4 Larval Development Studies

4:1 Introduction

Recent studies at the Kenya coast suggest that the introduced *C. partellus* may be gradually displacing the indigenous *C. orichalcociliellus* (Overholt *et al.*,1993). This study was designed to investigate one aspect of the biology of *C. partellus* and *C. orichalcociliellus* with the aim of providing an insight into any critical biological difference which may be influencing their relative abundance. The specific objective was to determine and compare the larval development period and number of instars for each species under laboratory conditions. Accurate identification of number of instars of pest insects is needed not only for this study but also for proper development of IPM. For example, the length of time an insect spends during its vulnerable life stages may affect the rates of attack by natural enemies, thereby influencing the overall population dynamics of the insect.

4:2 Materials and Methods

Larvae were reared individually in glass vials (7 cm x 2.5 cm) on artificial diet (Ochieng *et al.*, 1985) from eclosion to pupation at 28°C, 70 - 80% relative humidity, and a photophase of 12:12 (L:D). Larvae were inspected daily for signs of ecdysis. Cast head capsules from each instar except the last were measured using an ocular micrometer at 50x magnification. Measurement of the head capsules of the ultimate instar was not possible because the head capsules were usually split at ecdysis. The duration of each stadium was recorded. The data of each larva was kept separately to complete a record on the individual insect. Sex of the larvae was determined at pupation based on the position of the genital pores. In male pupae, the genital pore lies between a pair of raised cuticular lobes on the ninth abdominal sternite. In the female, the genital pore lies between the eighth and ninth abdominal sternites (Fig.2).

4:3 Data Analysis

The data was analysed according to species and sex. Means of head capsule size and larval development period were

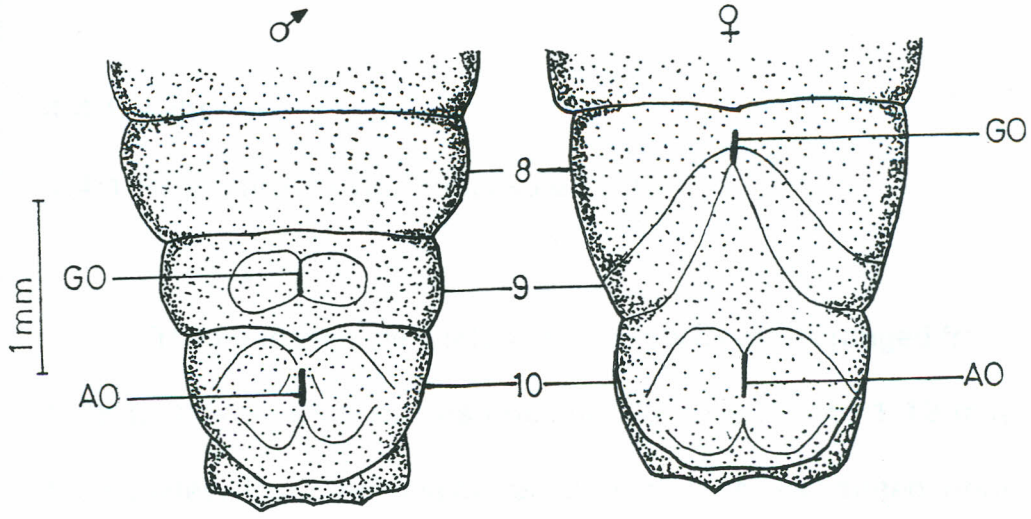

Fig.2: Ventral view of abdominal segments 8, 9 and 10 showing the distinction between male and female pupae of *Chilo partellus* and *C. orichalcociliellus*. AO, Anal opening; GO, Genital opening.

compared between species, and sexes within species, using analysis of variance (PROC GLM, SAS Institute, 1988). The means in each of the analyses were separated using either the t-test or the Student-Newman-Keuls (SNK) procedure (SAS, Institute, 1988).

4:4 Results

4:4:1 Head capsule width measurements

The head capsule widths of *C. partellus* males ranged from 0.28 to 1.10 mm for moults one and five and 0.28 to 1.13 mm for females. In *C. orichalcociliellus* head capsules ranged from 0.27 to 1.12 mm for males for moults one and six, and in females 0.27 to 1.31 for moults one and seven. The mean head capsule widths of *C. partellus* were significantly greater than *C. orichalcociliellus* (Table 1) for moults one to five as follows: moult 1, $t = 2.06$, $df = 41$, $P = 0.04$; Moult 2, $t = 3.49$, $df = 55$, $P = 0.001$; Moult 3, $t = 5.68$, $df = 113$, $P = 0.0001$; Moult 4, $t = 6.08$, $df = 115$, $P = 0.0001$; Moult 5, $t = 4.55$, $df = 102$, $P = 0.0001$. Although the mean head capsule widths were distinct for each moult apart from the first and second for the males in

each species (Table 1), variation in width at each moult resulted in overlap between consecutive moults. At each moult the mean head capsule size of the female and male were not statistically different except moult four in *C. partellus* (male significantly greater, $t = 3.11$, $df = 26$, $p = 0.005$) and moult six in *C. orichalcociliellus* (female significantly greater, $t = 3.47$, $df = 17$, $p = 0.003$).

4:4:2 Number of instars and stadial duration

The number of larval instars and mean duration of larval development for males and females of each species are presented in Table 2. In both species males had shorter developmental period and fewer mean number of instars as compared to the females. Table 3 presents the mean stadial periods for males and females for each instar and percentage of larvae pupating at each instar. Table 3 also shows the comparison of mean stadial periods between the sexes of the two species. Levels of significance at each instar were as follows: Males: first instar, $t = 2.24$, $df = 46$, $p = 0.03$; second instar, $t = 2.9$, $df = 18$, $p = 0.03$; third instar, $t = -0.22$, $df = 18$, $p = 0.12$; fourth instar, $t = 2.94$, $df = 34$, $p = 0.0009$; fifth instar, $t = 3.7$, $df = 49$, $p = 0.003$; sixth instar,

t= -3.3, df=56, p=0.001. Females: first instar, t= 3.6, df=36, p=0.001; second instar, t= -2.9, df=15, p=0.0004; third instar, t= -2.9, df=15, p=0.002; fourth instar, t= 0.62, df=21, p=0.06; fifth instar, t= 2.51, df=35, p=0.001; sixth instar, t= -0.26, df=35, p=0.16.

Table 4 presents the comparison of mean stadial periods for the males and females within a species. In some instars there were significant differences while in others the mean stadial period for males and females were not significantly different. Levels of significance for the different instars were as follows: *C. partellus*: First instar, means were the same; second instar, t= 1.51, df=16, p=0.1; third instar, t= 1.9, df=15, p=0.04, fourth instar, t= 1, df=15, p=0.5; fifth instar, t= 0.40, df=30, p=0.9; sixth instar, t= -2.1, df=29, p=0.003. *Chilo orichalcociliellus*: First instar, t= 3.01, df=49, p=0.03; second instar, t= -1.7, df=17, p=0.3; third instar, t= -0.97, df=14, p=0.6, fourth instar, t= -0.82, df=40, p=0.9; fifth instar, t= 0.34, df=54, p=0.23; sixth instar, t= 0.34, df=62, p=0.02; seventh instar, t= -1.01, df=18, p=0.3.

Table 1: Mean Head capsule widths for *Chilo partellus* and *Chilo orichalcociliellus* males and females at different moults.

	C. PARTELLUS			C. ORICHALCOCILIELLUS		
Moult	*Males* mm ± SD	*Females* mm ± SD	Mean for ♂&♀ mm±SD	*Males* mm ± SD	*Females* mm ± SD	*Mean for* ♂&♀ mm±SD
1	0.28 ±0.05 A	0.28 ± 0.05 A	0.28 ± 0.01b	0.27 ± 0.01 A	0.27 ± 0.02 A	0.27 ± 0.01a
2	0.36 ± 0.02 A	0.37 ± 0.03 B	0.37 ± 0.03b	0.34 ± 0.02 A	0.35 ± 0.05 B	0.34 ± 0.02a
3	0.53 ± 0.08 B	0.56 ± 0.07 C	0.55 ± 0.07b	0.48 ± 0.05 B	0.49 ± 0.08 C	0.48 ± 0.05a
4	0.88 ± 0.11 C	0.75 ± 0.09 D	0.83 ±0.12b	0.70 ±0.09 C	0.72 ±0.08 D	0.71± 0.8a
5	1.1 ± 0.12 D	1.13 ± 0.11 E	1.15 ±0.14b	0.99 ± 0.11 D	1.01 ± 0.12 E	1.01 ±0.12a
6	-	-	-	1.12 ± 0.12 E	1.38 ± 0.13 G	1.31±0.17
7	-	-	-	-	1.29±0.01 F	1.29 ± 0.01

Means followed by the same lower case letters(compares pooled means of both sexes) in the same row are not significantly different (t-test, $p<0.05$). Means followed by the same upper case letters in the same column are not significantly different (SNK $p<0.05$). See text for probability details.

Table 2: Duration of larval development and number of instars of *Chilo partellus* and *Chilo orichalcociliellus* males and females.

Sex	Species	Mean Duration of Larval Development	N	Mean Numbers of Instars	N
Males	*C. partellus*	23.7a	34	5.3a	30
	C. orichalcociliellus	31.0b	43	6.1b	41
Females	*C. partellus*	26.6a	22	5.8a	19
	C. orichalcociliellus	37.9b	38	6.7b	29

Means followed by the same letters in the same column and sex are not significantly different, t-test, $p<0.05$.

Table 3: Mean stadial periods in days for *C. partellus* and *C. orichalcociliellus* males and females and percentage number of larvae pupating after each instar.

	MALE				FEMALE			
Larval Instar	*C. partellus*		*C. orichalcociliellus*		*C. partellus*		*C. orichalcociliellus*	
	DAYS ± SD	% PUPATING	DAYS ± SD	% PUPATING	DAYS ± SD	% PUPATING	DAYS ± SD	% PUPATING
First	5 ± 0.0 b	-	4.8 ± 0.4 a	-	5.0 ± 0.0 b	-	4.4 ± 0.6 a	-
Second	2.3 ± 0.5 a	-	3.3 ± 1.0 b	-	2.0 ± 0.0 a	-	4.3 ± 1.5 b	-
Third	3.7 ± 0.8 a	-	4.0 ± 1.5 a	-	3.1 ± 0.3 a	-	4.1 ± 1.1 b	-
Fourth	5.1 ± 2.0 b	-	3.3 ± 0.8 a	-	3.9 ± 1.5 a	-	3.6 ± 0.8 a	-
Fifth	7.0 ± 2.6 b	70.0	4.9 ± 1.4 a	-	6.6 ± 2.6 b	16.0	4.8 ± 1.8 a	-
Sixth	6.7 ± 2.0 a	30.0	8.0 ± 2.4 b	87.8	7.8 ± 2.5 a	84.0	7.7 ± 3.6 a	37.9
Seventh	-	-	9.6 ± 2.9	12.2	-	-	12.1 ± 5.1	55.2
Eighth	-	-	-	-	-	-	9.5 ± 6.4	6.9

Means followed by the same letters in the same row and sex are not significantly different (t-test $P<0.05$) see text for levels of significance for each instar.

Table 4: Comparison of mean stadial periods in days for males and females of *C. partellus* *C. orichalcociliellus*.

	C. PARTELLUS		*C. ORICHALCOCILIELLUS*	
Larval Instar	Males	Females	Males	Females
	DAYS ± SD	DAYS ± SD	DAYS ± SD	DAYS ± SD
First	5 ± 0.0 a	5.0 ± 0.0 a	4.8 ± 0.4 a	4.4 ± 0.6 b
Second	2.3 ± 0.5 a	2.0 ± 0.0 a	3.3 ± 1.0 a	4.3 ± 1.5 a
Third	3.7 ± 0.8 a	3.1 ± 0.3 b	4.0 ± 1.5 a	4.1 ± 1.1 a
Fourth	5.1 ± 2.0 b	3.9 ± 1.5 a	3.3 ± 0.8 a	3.6 ± 0.8 a
Fifth	7.0 ± 2.6 a	6.6 ± 2.6 a	4.9 ± 1.4 a	4.8 ± 1.8 a
Sixth	6.7 ± 2.0 a	7.8 ± 2.5 b	8.0 ± 2.4 b	7.7 ± 3.6 a
Seventh	-	-	9.6 ± 2.9 a	12.1 ± 5.1 a
Eighth	-	-	-	9.5 ± 6.4

Means followed by the same letters in the same row within a species are not significantly diffe
t-test $P < 0.05$). See text for the different probability levels for each instar.

4:4:3 Larval development period

The mean larval developmental periods for males and females of each species are shown in Table 2. The mean duration for larval development was shorter in males than females in both species. The mean larval duration was longer in *C. orichalcociliellus* than in *C. partellus* for both sexes. Overall mean larval development period was 24.9 days for *C. partellus* and 34.3 days for *C. orichalcociliellus*.

4:5 Discussion

The number of instars and head capsule sizes for *C. partellus* observed in the present study are generally in agreement with earlier publication (Ampofo, 1988), but differ from Alghali who reported the presence of only five instars (Alghali,1985). The head capsule widths of *C. orichalcociliellus* were similar to those reported by Delobel (1975b) for instars one to four but slightly smaller than the sizes he reported for instars five and six. Lack of clear separation between head capsules of different instars indicated that the larvae were quite heterogenous in their rate of development.

Larvae of both species exhibited sexual dimorphism with most females completing more instars than males (Table, 2). Indeed, both species exhibited developmental polymorphism with larvae of the same species and same sex completing different numbers of instars. These observations were in line with those of Schmidt and Lauer (1977) and Schmidt *et al.*, (1977) who reported similar developmental polymorphism in some tortricids (Lepidoptera: Tortricidae). Developmental polymorphism has previously been reported in *Chilo* spp. (Ampofo, 1988; Scheltes, 1978; Mathez, 1972; Delobel, 1975b).

It is interesting to note that the mean width for head capsule seven in *C. orichalcociliellus* was less than the width of capsule six. This suggested that the larvae cease to grow after the seventh instar. It was observed that older larvae constructed silken chambers within the diet and remained there with little or no feeding at all. Similar aestivation/diapause behaviour has been previously reported for *C. partellus* (Mathez, 1972; Scheltes, 1978; Ampofo, 1988) and *C. orichalcociliellus* (Delobel, 1975b). Scheltes (1978) recorded that the mean width of the larval head capsules sometimes tended to decrease when diapause became intense in *C. partellus*.

The difference in larval developmental periods between the two species would result in an overall shorter generational time for *C. partellus* if the egg, pupal, and preovipositional periods of *C. partellus* and *C. orichalcociliellus* were approximately the same, which appears to be the case. The egg developmental period for *C. partellus* is four to five days at 26-28°C (Berger, 1989) and five to six days for *C. orichalcociliellus* at 28°C (Delobel, 1975b). The pupal stage lasts four to eight days in *C. partellus* (Neupane *et al.*, 1985) and six to nine days in *C. orichalcociliellus* (Delobel, 1975b). In both species oviposition begins on the night of emergence and continues for two nights (Berger, 1989; Delobel, 1975b). Therefore, it appears that *C. partellus* is capable of completing more generations than *C. orichalcociliellus* in the same amount of time. A total generational time of 25 - 50 days has been reported for *C. partellus* (Harris, 1990; Ingram, 1958; Neupane *et al.*, 1985), and 38-57 days for *C. orichalcociliellus* (Delobel 1975b). The fecundity of the two species is approximately equal with *C. partellus* ovipositing a mean of 434 eggs (Berger, 1989), and *C. orichalcociliellus* ovipositing a mean of 475 eggs (Delobel, 1975b).

During the time that *C. partellus* has invaded the coastal area of Kenya, it has become the major stemborer pest of maize

and sorghum, surpassing in importance the indigenous *C. orichalcociliellus* (Overholt *et al.*, 1993). A similar phenomena occurred in Madagascar, where *C. partellus* was first reported in 1972, but within a short period of three years, *C. partellus* had become a more economically important pest than *C. orichalcociliellus* (Delobel, 1975b). The observations in this study suggested that the shorter generational time of *C. partellus* could explain the superior competitiveness of *C. partellus* over the indigenous *C. orichalcociliellus*. Since larvae in this study were fed on artificial diet developed for *C. partellus* (Ochieng *et al.*, 1985), further investigation should be conducted using the natural host plants to see if similar results could be obtained.

CHAPTER FIVE

5 Morphological Studies

5:1 Introduction

There is need for studies to identify reliable characters for the distinction of the larval stages of *C. partellus* and *C. orichalcociliellus*. This part of the study aimed at evaluating external morphological characters for the purpose of separating the larvae of these two species.

5:2 Materials and Methods

Larvae were removed from the 70% alcohol and the number of crochets on both caudal and abdominal prolegs and the shape of spiracles recorded. The tubercles on the abdomen and thorax were also drawn and compared. For the chaetotaxy (arrangement of larval setae on the body), and mandibular structure study, larvae were prepared by separating the head capsule from the body, then macerating the parts in 10% potassium hydroxide overnight. Skin preparations were made using the method of

Hinton (1956) of slitting the body laterally so as to preserve the chaetotaxy of one side. The integument was then mounted on a microscope slide in glycerol and the thoracic and abdominal setae of each segment drawn. For a proper understanding of the chaetotaxy, it was necessary to examine each of the segments. Setae were named using Hinton's classification (Hinton, 1946) which defines body setae as dorsal, sub-dorsal,lateral, sub-lateral, ventral and includes the microscopic setae (Fig.3). Mandibles were detached from the head capsule and were dehydrated by placing them in 70%, 80% and 100% ethanol.The dry specimens were mounted on copper stubs with double-sided sticky tape on scanning electron microscope (SEM) copper stubs. The stubs were sputter coated with a layer of gold-pallidium using a Fine Coat ion sputter (JFC-1100). The specimens were observed on a Jeol Stereoscan (JSM-T1000) and micrographs were taken from both species and compared.

5:3 Data analysis

The number of crochets of the two species on abdominal and caudal prolegs were compared with t-tests (SAS Institute, 1988).

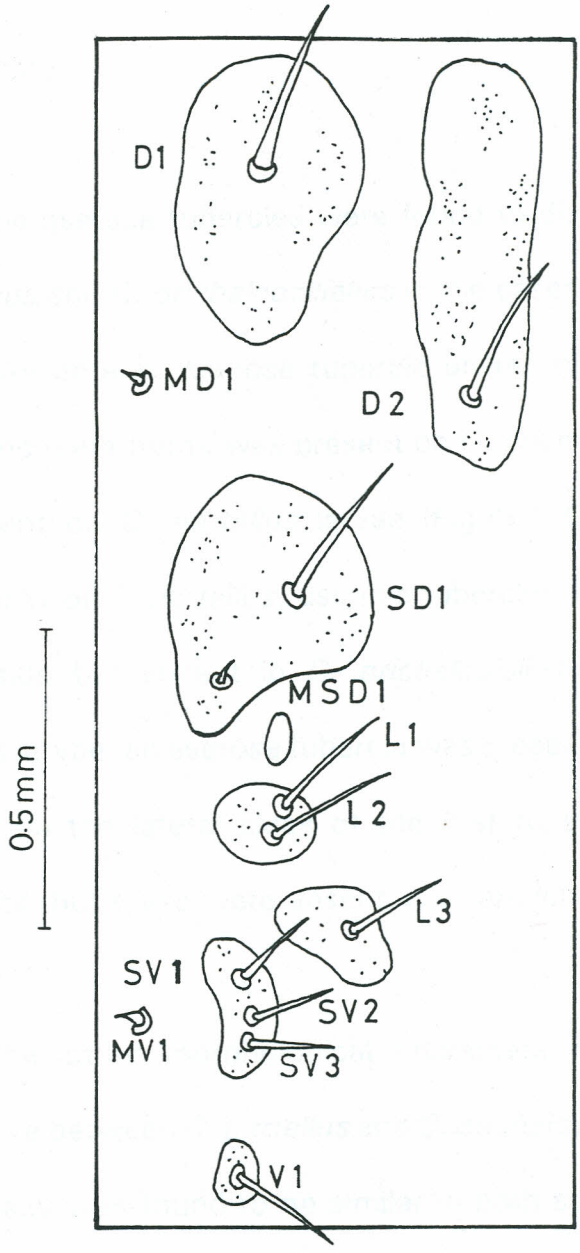

Fig.3: Nomenclature of the larval body chaetotaxy. (D,dorsal; SD, sub-dorsal; L, lateral; V, ventral; SV, sub-ventral; MD, microscopic dorsal; MV, microscopic ventral).

5:4 Results

The asetose tubercles were found to distinguish between *C.partellus* and *C. orichalcociliellus* in the cases where they were visible. An anterior asetose tubercle on the dorsal sides of the meso- and metathorax was present on *C. orichalcociliellus* larvae but absent on *C. partellus* larvae (Fig.4). On the meso and metathorax of *C. partellus*, asetose tubercles are present on the lateral side but absent in *C. orichalcociliellus* (Fig.4). In *C. partellus* larvae, an asetose tubercle was present posterior to each spiracle on the lateral sides of the first to seventh abdominal segments, but these were absent in *C. orichalcociliellus* (Fig.4).

The other morphological characters studied were not distinctive between *C. partellus* and *C. orichalcociliellus*. The body chaetotaxy was found to be similar in both species (Fig.5). The spiracles were also alike being oval and open. The mandibles bore six protuberances in the form of teeth and two setae (Fig. 6). In both species, abdominal prolegs were of the circular type (Fig.7) with varying numbers of crochets (Table 5). The caudal prolegs were of the semicircular type (Fig.7) with varying number of crochets (Table 5). There was no significant difference in the

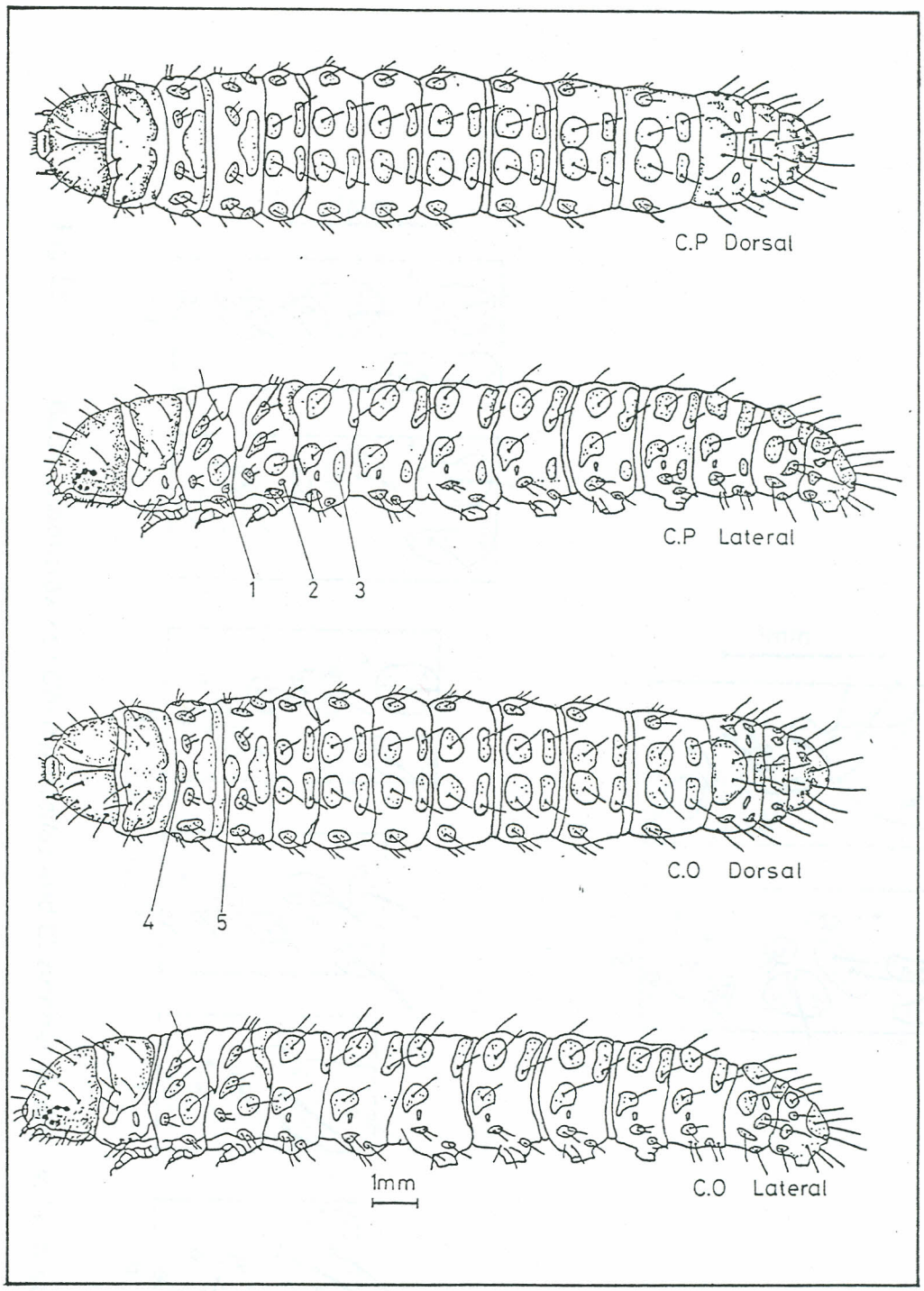

Fig.4: Diagrammatic presentation of the distinguishing asetose tubercles (1-5) on *Chilo partellus* and *C. orichalcociliellus* larvae.

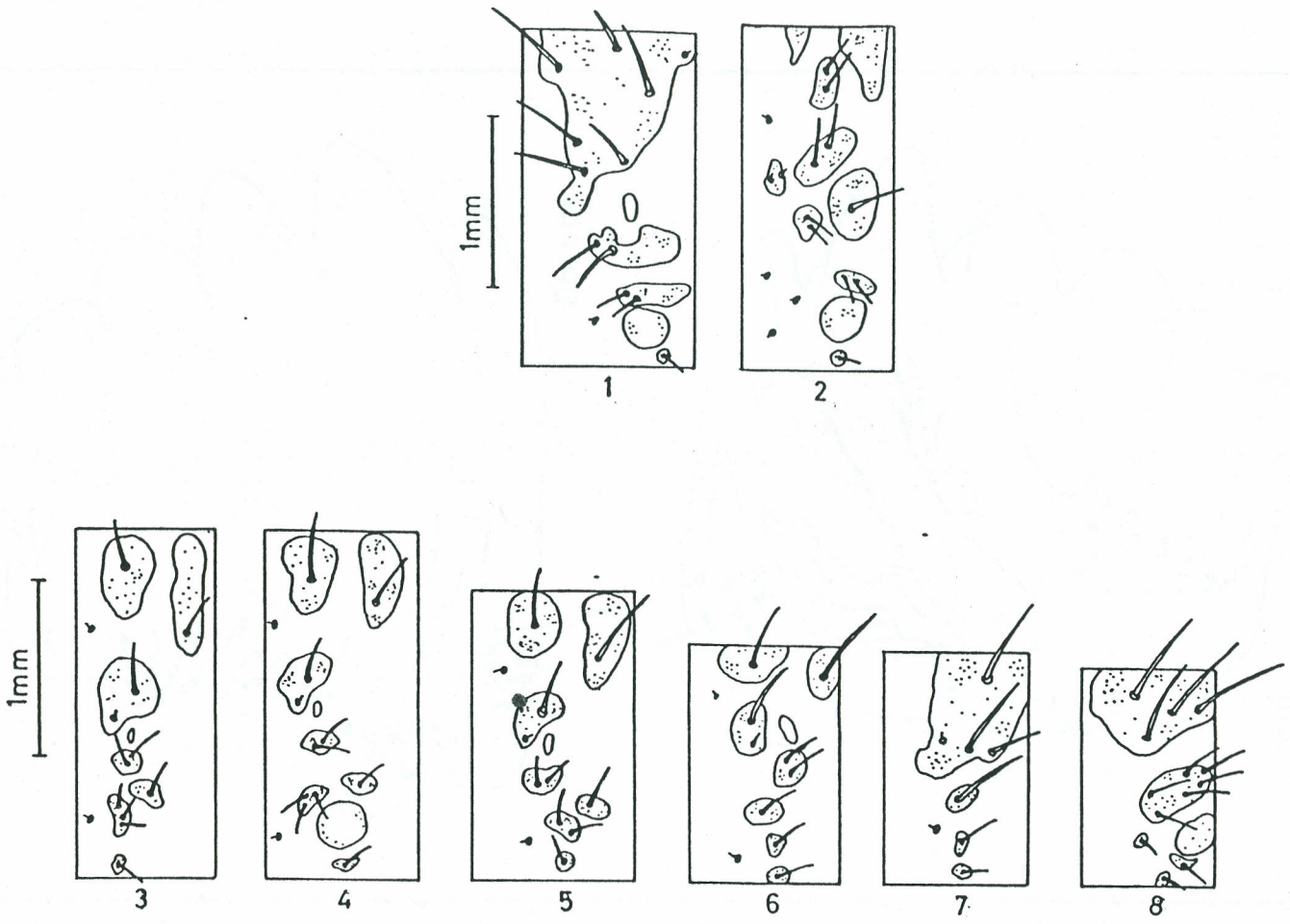

Fig.5: Body chaetotaxy of *Chilo partellus* and *C. orichalcociliellus*. (1, first thoracic segment; 2, second and third thoracic segments; 3, abdominal segments 1&2; 4, segments 3-6; 5, segment 7; 6, segment 8; 7, segment 9; 8, segment 10).

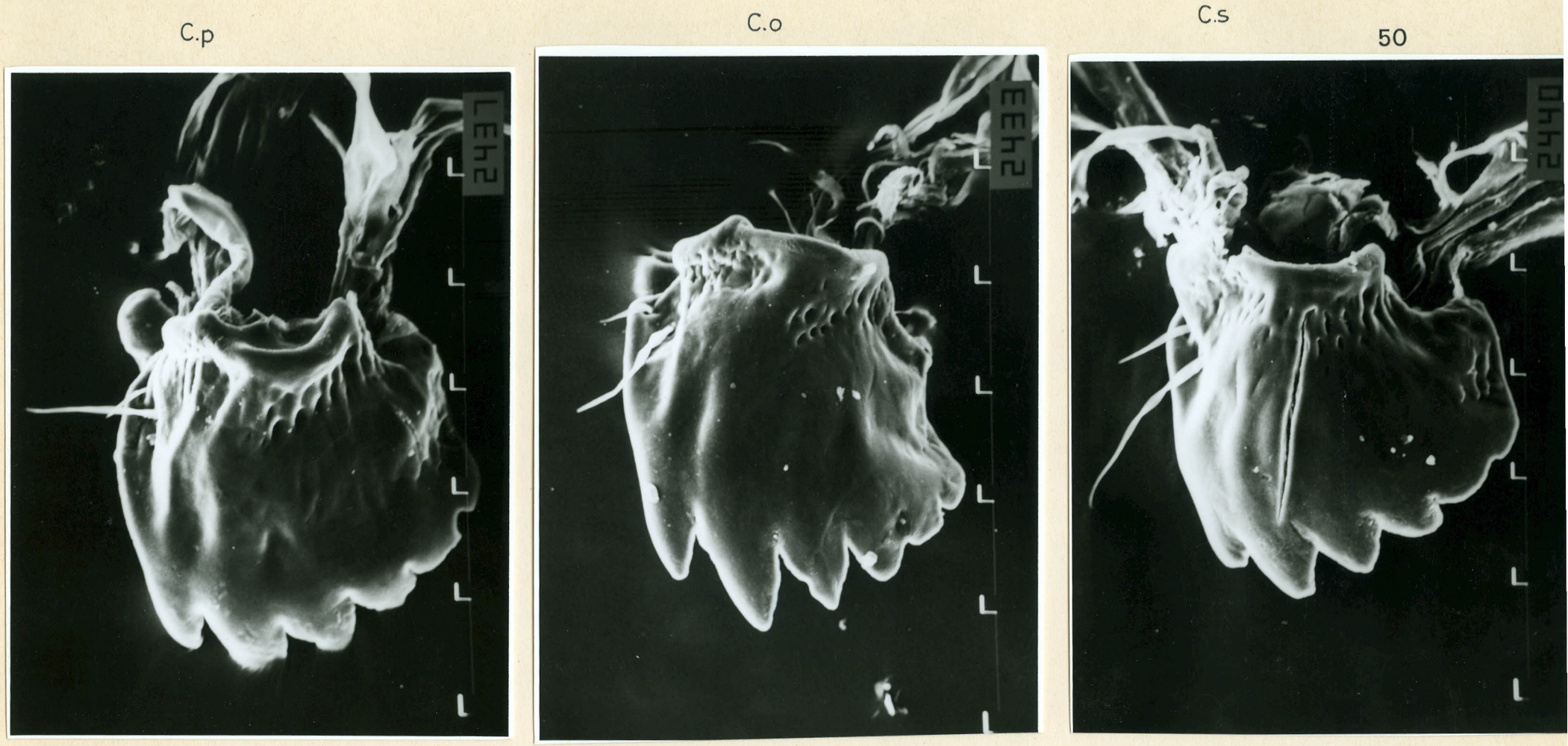

Plate 1: Scanning electron microscope micrographs of the mandibles of *Chilo partellus* (C.p) and *C. orichalcociliellus* (C.o) and aestivating *Chilo* spp. (C.s) (dorsal view at magnification x200; WD, 20; kV, 25).

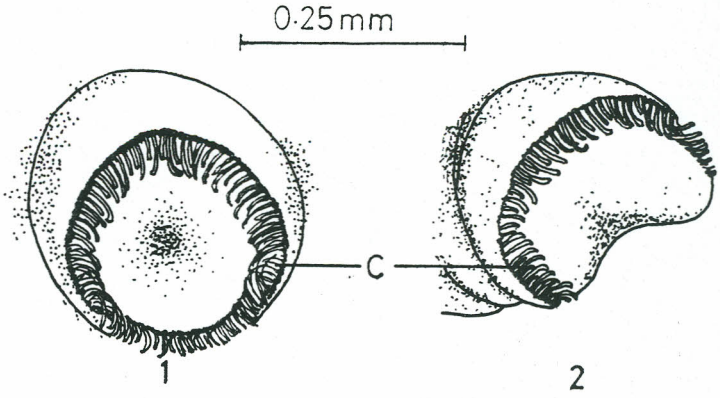

Fig. 7: Diagrammatic presentation of abdominal (1) and caudal (2) prolegs of *Chilo partellus* and *C. orichalcociliellus*. (c, crochets).

mean number of crochets on abdominal prolegs ($t = 1.09$, $df = 28$ $p = 0.15$) and mean number of crochets on caudal prolegs ($t = 1.61$, $df = 28$, $p = 0.5$) between the two species.

Table 5: Variation and mean number of crochets on abdominal and caudal prolegs of *C. partellus* and *C. orichalcociliellus* larvae.

Species	Variable	N	Minimum	Maximum	Mean	SD
C. partellus	Abdominal	15	31	49	41.3	5.6
C. orichal.	Abdominal	15	30	59	44.5	6.7
C. partellus	Caudal	15	20	31	25.0	0.2
C. orichal.	Caudal	15	20	32	25.8	3.7
Aestivating	Abdominal	4	38	44	40.8	2.8
Aestivating	Caudal	4	20	25	21.8	2.2

The mean number of crochets on either abdominal or caudal prolegs did not show significantly difference (t-test, $p<0.05$).

5:5 Discussion

The general form of caterpillars has been widely employed since the eighteenth century in classifying the Lepidoptera (Kitching, 1984). Heinrich (1916) was probably the first to recognize the potential of such characters as setae, mouthparts and legs as sources of taxonomic characters of larvae. Hinton (1956) found mandibles of tineids (Lepidoptera: Tineidae) quite distinct, those of *Acedes pallescentella* Stainton being narrowed apically while in *A. fuscipunctella* Haworth the mandibles were quadrate and not narrowed apically.

In this study, no such clear characters were found that could be reliably used to distinguish *C. partellus* larvae from those of *C. orichalcociliellus*. The body chaetotaxy was also found to be similar in both species as was previously reported by Mathez (1972). Similar reports were made by Kitching (1984) who found that chaetotaxy was of greater use at the generic level and above than at the specific level in danaines (Lepidoptera:Nymphalidae).

Spiracles have been mentioned as sources of taxonomic characters by Isaac and Rao (1941) and Gupta (1940). In this

study, the spiracles of *C. partellus* and *C. orichalcociliellus* were similar being oval and not fully closed. Similar findings were reported for *C. partellus* by Gupta (1940) but Isaac and Rao (1941) reported the spiracles of *C. partellus* as closed. The type of crochets on prolegs have been used by several workers for taxonomic purposes (Nye, 1960; Mathez, 1972; Holloway *et al.*, 1987). In this study, the shape, arrangement and number of crochets on both abdominal and caudal prolegs were not found to be distinctive between the two *Chilo* species. There was variation in numbers of crochets in both species and the ranges for the two species overlapped. An overlap in the number of crochets in these two species has also been reported by Delobel (1975a). The overlap and lack of consistency in the number of crochets does not allow for their effective application in the separation of *C. partellus* and *C. orichalcociliellus*. Since the tubercles can only be used to distinguish larvae with pigmentation, further studies on biochemical characters are warranted.

CHAPTER SIX

6 Analysis of Cuticular Components

6:1 Introduction

Cuticular component analysis has been used by some workers to differentiate individuals that appear morphologically similar. Specimens are rinsed with non-polar solvents, and the wash is analysed by gas chromatography (GC). The objective of this part of the study was to analyse hexane extracts of cuticular components using GC to see if their composition provided new characters for the separation of *C. partellus* and *C. orichalcociliellus* larvae.

6:2 Materials and Methods

6:2:1 Sample preparation

Large larvae (fifth instars and above) were used for cuticular component analysis of the following groups: 1. *Chilo partellus* from the field; 2. *C. partellus* from the laboratory; 3.

C.orichalcociliellus from the field; 4. *C. orichalcociliellus* from the laboratory; 5. *C.orichalcociliellus* aestivating from the laboratory; and, 6. *Chilo* species aestivating from the field. The larvae were removed from the freezer and left at room temperature to thaw for fifteen minutes. Larvae were then placed individually in a pyrex tube and washed in 200 microlitres of redistilled hexane in which 0.003 % n-tetracosane had been added as an internal standard. The washing (extraction) lasted for five minutes after which the sample extract was concentrated by evaporating under a stream of nitrogen to five microlitres.

6:2:2 Gas chromatographic separation

The five microlitres of the extract was injected into a Hewlett Packard (HP) 5890 A series gas chromatograph, fitted with a flame ionization detector (FID). The FID responds to compounds which produce ions when burned in a hydrogen-air flame. The gas chromatograph was fitted with a 50 m Hewlett Packard fused silica capillary column, cross linked with methyl silicone with an internal diameter of 0.31 mm.

The HP 5890 A was programmed as follows, initial oven temperature of 100°C for 5 minutes, then increased to 220°C at the rate of 10°C per minute. From 220°C the rate of increase was 5°C per minute up to a final temperature of 280°C. There was a hold of 20 minutes at the final temperature. This programme was determined after several trials to get a standard programme giving the best peak separation. The injector temperature was 230°C and the detector temperature 280°C. Nitrogen was used as the carrier gas and hydrogen as fuel for the FID. A Hewlett Packard 3393 A computing integrator was used to determine the peak area, percentage peak area and peak heights of the samples.

6:2:3 Data analysis

The data on percentage peak area were subjected first to analysis of variance to determine whether the percentage area of single peaks could discriminate between the six groups mentioned in section 6:2:1. Secondly, the data were subjected to canonical discriminant analysis (PROC CANDISC, SAS Institute, 1988). This procedure uses a classification variable and several quantitative variables to derive canonical variables. The canonical variables are a combination of the quantitative variables that

summarise the variation between the classes. The results of the analyses were used to produce plots of the data from individual samples for the first and second canonical variables for simple visual interpretation of relationships.

6:3 Results

There were no qualitative differences between the profiles of the two species but quantitative differences were observed between the peak percentage areas. The ten most consistent peaks for all the groups were selected (Fig.8) and their peak percentage areas were used for further analysis. The percentage areas of the peaks were analysed to determine if field and laboratory individuals of the same species had similar cuticular components, and secondly if the six groups could be reliably separated into their respective species.

Table 6 gives the results obtained after comparison of means for all the peaks. Some peaks showed no differences among the six groups of larvae while other peaks showed significant differences (Table 7). Canonical variates based on peak area percentage did not provide discrimination between the

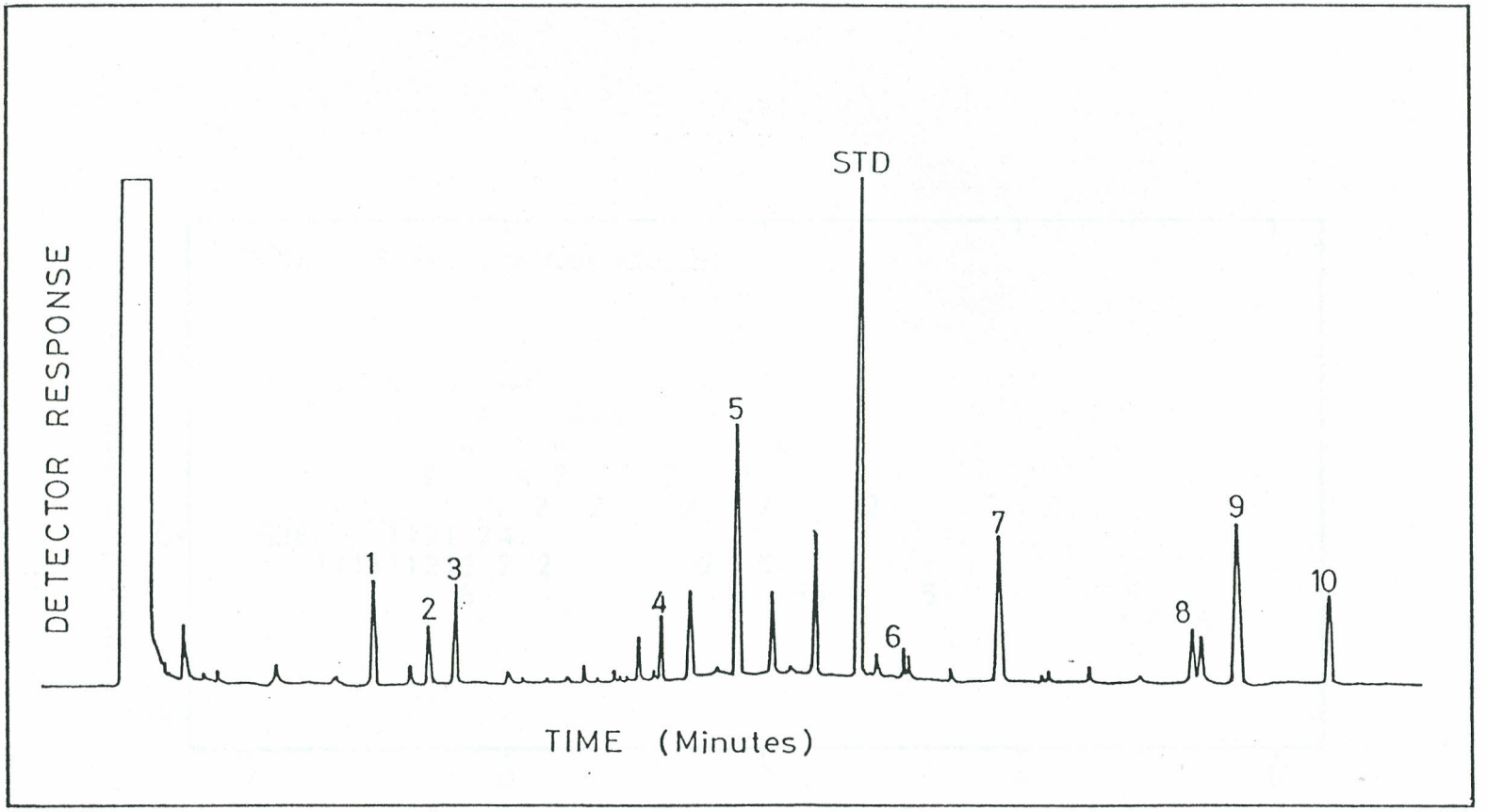

Fig. 8 : GC cuticular component chromatogram showing the ten peaks selected for analysis. Retention time for each peak is shown in table 7.
STD = Peak for n-tetracosane

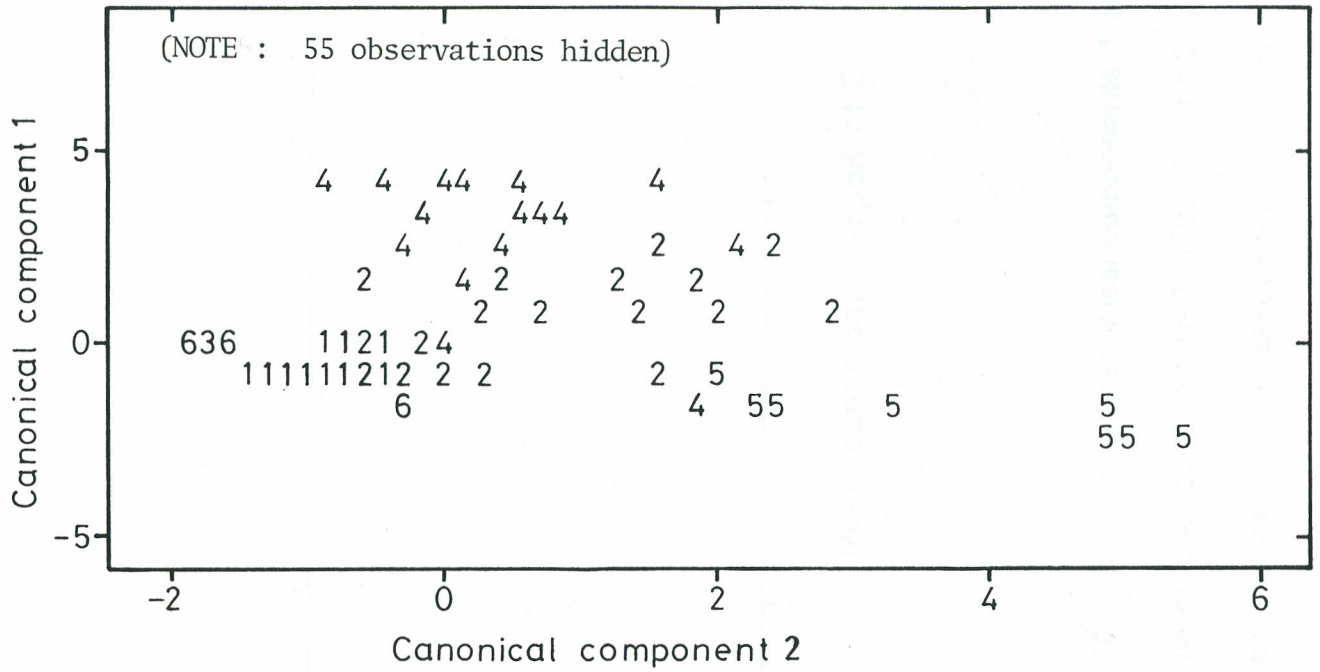

Fig. 9: Plot of canonical components 1 and 2 for the six sample groups (*C. partellus*- 1, field; 2, lab; *C. orichalcociliellus*- 3, field; 4, lab; 5, lab. aestivating; 6, unidentified aestivating larvae from field).

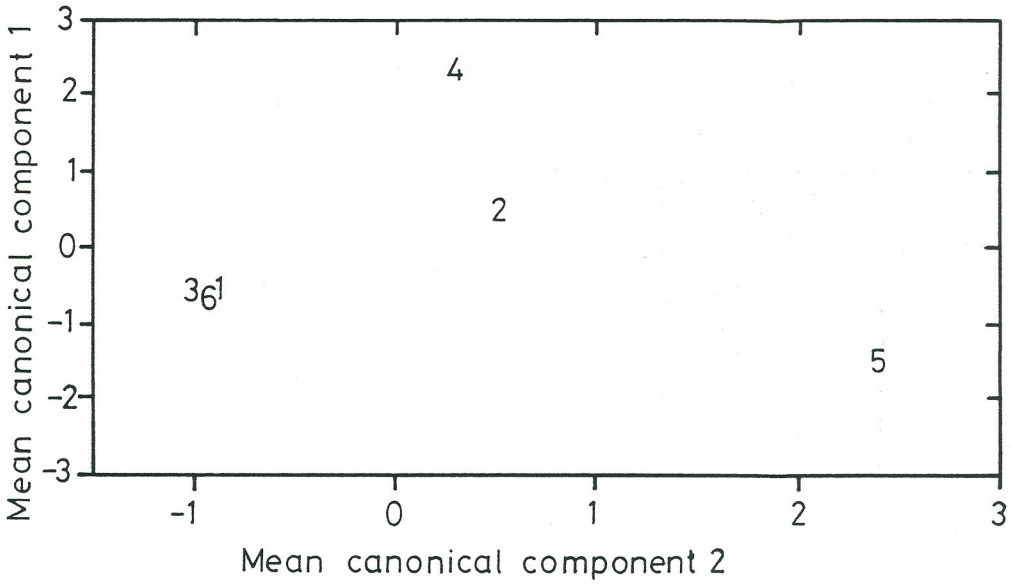

Fig. 10: Plot of mean canonical components 1 and 2 for the six sample groups (*C. partellus*- 1, field; 2, lab; *C. orichalcociliellus*- 3, field; 4, lab; 5, lab. aestivating; 6, unidentified aestivating larvae from field).

species (Fig.9), but supported what had been shown by comparison of the means above. When the means of the canonical variates were plotted (Fig. 10), the field specimens of the two species clustered together although those of the laboratory specimens were separate.

Table 6: Comparison of total area percentage of ten cuticular component peaks.

Species	N	Mean area percentage
C. partellus (Field)	200	0.88a
C. partellus (Lab)	230	1.03b
C. orichalcociliellus (Field)	150	0.90a
C. orichalcociliellus (Lab)	200	1.04b
C. orichalcociliellus (Lab. aestivating)	140	1.00b
Chilo species (Field aestivating)	190	0.89a

Means followed by the same letters are not significantly different. (T-test (LSD), $p < 0.05$).

Table 7: Comparison of mean area percentage for ten cuticular component peaks.

Peak No.	Retention time	*C. partellus* Field N = 20	*C. partellus* Lab. N = 23	*C. orichalcociliellus* Field N = 15	*C. orichalcociliellus* Lab. N = 20	*C. orichalcociliellus* Lab(A*) N = 14	*Chilo* spp. Field(A*) N = 19
1	9.52	0.83b,c	1.14a	0.86b	1.05a	0.71c	0.81b,c
2	10.91	0.87b	1.16a	0.88b	1.18a	0.88b	0.82b
3	11.63	0.83b	1.12a	0.78b	1.12a	1.19a	0.76b
4	17.66	0.92b	1.19a	0.93b	1.15a	1.16a	0.95b
5	19.77	1.45a,b	1.19b,c	1.62a	1.01c	1.46a,b	1.54a
6	24.67	0.92b	0.89b	0.93b	0.97b	1.23a	0.88b
7	27.05	0.74b	0.79b	0.77b	0.81b	1.11a	0.81b
8	32.65	0.76b	0.92a	0.82a,b	0.89a	0.89a	0.81a,b
9	34.05	0.71c	1.09b	0.71c	1.43a	0.71c	0.74c
10	36.78	0.76a	0.76a	0.71a	0.75a	0.72a	0.75a

Means followed by the same letters in the same row are not significantly different, t-test (LSD), $p < 0.05$.

*(A) stands for aestivating larvae

6:4 Discussion

Cuticular component analysis of *C. partellus* and *C. orichalcociliellus* larvae was not successful in distinguishing between the two species. The peak patterns of the two species were qualitatively similar. These observations are similar to what was reported in two *Anopheles* Meigen species (Carlson *et al.*, 1981). The results from the statistical analysis indicated the existence of a close relationship among larvae from the same environment irrespective of their species. *Chilo partellus* and *C. orichalcociliellus* are ecologically similar species and occur sympatrically at the sample sites. In the laboratory, the two species were reared on the same diet under similar controlled conditions. The diet may have influenced the composition of the cuticular components of these two stemborer species, thus the similarity of *C. partellus* and *C. orichalcociliellus* fed on same diet. A correlation between the composition of the lipids from an insects cuticle and that of its diet has been previously reported by Espelie and Bernays (1989), Espelie and Brown (1990), Baker *et al.* (1978), Richter and Krain (1980) and Blomquist and Jackson (1973) among others.

It is also possible that the environmental conditions in the field and in the laboratory influenced the amount of cuticular components synthesised by the larvae resulting in larvae of *C.partellus* and of *C. orichalcociliellus* from similar environment having similar quantity of cuticular components. Armold and Regnier, (1975) reported that cuticular hydrocarbons were of major importance in a wide range of ecological roles and their quantity, and at times quality, were correlated with the need to conserve water. Hadley (1977) reported that the hydrocarbon composition of the tenebrionid beetle *Eleodes armata* (Lec) (Coleoptera: Tenebrionidae) varied depending on season. Changes in cuticular hydrocarbons related to environmental conditions were also reported in the flesh fly, *Sarcophaga bullata* (Haag) (Diptera: Sarcophagidae) (Armold and Regnier, 1975). In this respect, the aestivating larvae were expected to have had a higher quantity of the cuticular components, but this was not observed in this study. The results obtained here suggested that cuticular component analysis as applied in this study may not have been the best tool for taxonomic distinction of *C. partellus* and *C. orichalcociliellus* larvae.

CHAPTER SEVEN

7 Starch Gel Electrophoresis

7:1 Introduction

Gel electrophoresis is a widely used biochemical technique in insect systematics (Blackman and Spence, 1992). With the success so far achieved in a wide variety of plant and animal species (Britton-Davidan and Amourex, 1982), it is hoped that enzyme analysis will provide reliable biochemical characters for the separation of *C. partellus* and *C. orichalcociliellus* larvae.

7:2 Materials and Methods

7:2:1 Gel and electrode buffer systems

The three buffer systems used in this study are presented below while Table 8 shows the enzyme systems studied and the buffer systems used.

Table 8: Enzyme systems analyzed in *C. partellus* and *C. orichalcociliellus* larvae, enzyme classification (E.C) number (IUPAC,1973) and the gel-electrode buffer systems used in their analysis. See text for description of the buffer systems.

Enzyme system	E.C. No.	Buffer system
Alpha-glycerophosphate dehydrogenase	1.1.1.8	TC
Glutamate oxaloacetate transaminase	2.6.1.1	CA8
Hexokinase	2.7.1.1	CA8
Glucose-6-phosphate	1.1.1.49	TC
Malic enzyme	1.1.1.40	TC
Isocitric dehydrogenase	1.1.1.42	TC
Malate dehydrogenase	1.1.1.37	TC
Alcohol dehydrogenase	1.1.1.1	CA8
Lactate dehydrogenase	1.1.1.27	CA8
Phosphogluco mutase	5.4.2.2	TM
Esterase	3.1.1.1	TC
Glucose phosphate isomerase	5.3.1.9	TC
Aldolase	4.1.2.13	CA8

1. CA-8 buffer system: Gel buffer: 0.074 M Tris and 0.009 M citric acid, pH adjusted to 8.55. Electrode buffer: 1.37 M Tris and 0.314 M citric acid, pH adjusted to 8.15, (cathode tray buffer was diluted 1:4 with distilled water and anode tray 1:5) (Steiner and Joslyn, 1979).

2. Tris maleate (TM) pH 7.4 for both gel and electrode buffers: 0.1 M maleic acid, 0.013 EDTA and 0.01 M $MgCl_2$. The gel buffer was diluted 1:10.

3. Tris- Citrate (TC) buffer: 0.22 M Tris and 0.08 M citric acid. Electrode buffer was adjusted to pH 6.3 and gel buffer to pH 6.7. The gel buffer was diluted 1:14 before use (Pasteur *et al.*, 1988).

7:2:2 Gel preparation

Gels were prepared by using 50 ml of gel buffer and 3.8 grammes of starch (Sigma S-4501). The starch was dissolved in the gel buffer and heated to boiling in a conical flask on a magnetic stirring plate. The solution was then degassed under vacuum and poured into a 22x15 cm glass plate. The gel was covered to prevent contamination and left to harden for at least

three hours.

7:2:3 Sample preparation

Portions of medium and large larvae were individually homogenized in 20 microlitres of 0.1 M Tris- HCl, PH 7.4 buffer in wells on glass plate using a homogenizing rod (in the case of small larvae,the whole body was homogenized). This was done on ice to minimize denaturing of the enzymes. Cotton threads 5 mm long, previously boiled for five minutes and dried, were soaked in the crude homogenate and inserted into slots made on the gel by a perspex comb. On each end of the gel, cotton threads soaked in bromophenol blue were used as a front running marker.

7:2:4 Running conditions of samples on starch gel

The glass plate containing the gel was placed on a flat bed apparatus (FBE 3,000, Pharmacia, Sweden). The temperature was maintained at 4.3°C using a hetofrig cooler (Heto Bikerod, Denmark). Sponge wicks were placed on the gel so that the current could flow from the cathode to anode. The gels were covered with glass plates which also held the wicks on to the

gels. The current was supplied at a constant 250 V for two and a half hours. After the run was completed, the gels were removed from the flat bed apparatus and stained for specific enzymes using modified histochemical methods outlined by Harris and Hopkinson (1978) and Pasteur *et al.,* (1988). The modified recipes are shown in Appendix 1.

7:2:5 Data recording and analysis

Bands were drawn immediately after staining. Loci and bands were designated beginning from the anodal end of the gel according to their mobility. The most anodal locus was designated (1) and the most anodal band (a). The rest of the loci and bands were designated numbers or letters depending on their mobilities relative to the (1) or (a) respectively.

7:3 Results

The enzyme systems assayed in this study fall into three categories:

(i) those which produced clear diagnostic bands able to distinguish *C.partellus* and *C. orichalcociliellus* larvae. These included Glucose phosphate isomerase (GPI), Lactate dehydrogenase (LDH) and Glutamate oxaloacetate transaminase (GOT);

(ii) those that did not produce clear bands but were characterised by a great amount of smearing. These included Alpha-glycerophosphate dehydrogenase (alpha-GPD), Hexokinase (HK), Glucose-6-phosphate (G6PD), Aldolase (ALD) and Esterase (EST), and,

(iii) those that produced clear bands but were not qualitatively different between the two species. These included Malic enzyme (ME), Isocitric dehydrogenase (IDH), Malate dehydrogenase (MDH), Alcohol dehydrogenase (ADH) and Phosphogluco mutase (PGM).

It was also observed that the very young larvae did not stain in a number of cases when one cotton thread was used. When a number of these young larvae from the laboratory colonies were homogenized together there was no problem with staining. The results of the first category of enzymes which gave diagnostic bands between the larvae of *C. partellus* and those of *C. orichalcociliellus* are shown in Figures 11, 12, 13 and in Table

9. *Chilo partellus* larvae had slow migrating bands for GPI and LDH while *C. orichalcociliellus* had faster migrating bands for these two enzymes. The two GOT loci of *C. partellus* showed polymorphisms whereas the *C. orichalcociliellus* had only one GOT locus which did not show variation. Of the total 778 larvae assayed for these three diagnostic enzymes, 447 were from the field and were found to consist of 73.2% *C. partellus* and 26.8% *C. orichalcociliellus*.

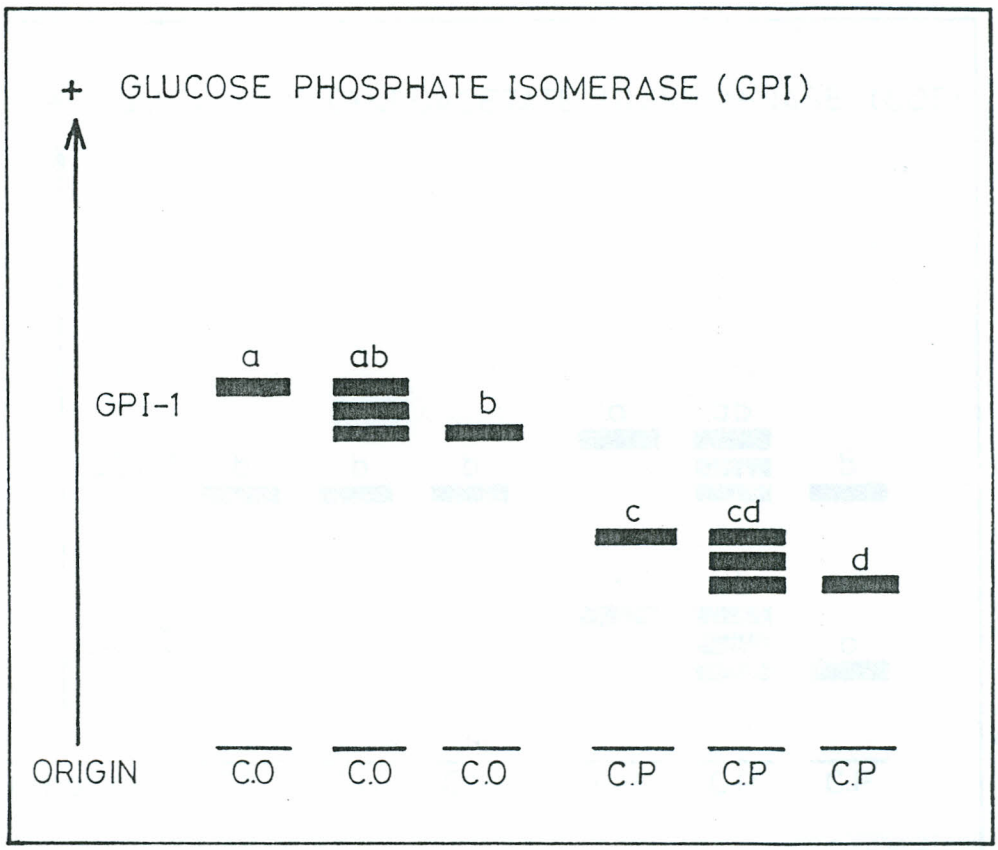

Fig. 11: Glucose phosphate isomerase band patterns for C. orichalcociliellus (C.O) and C. partellus (C.P) (+) denotes the anodal end of the gel.

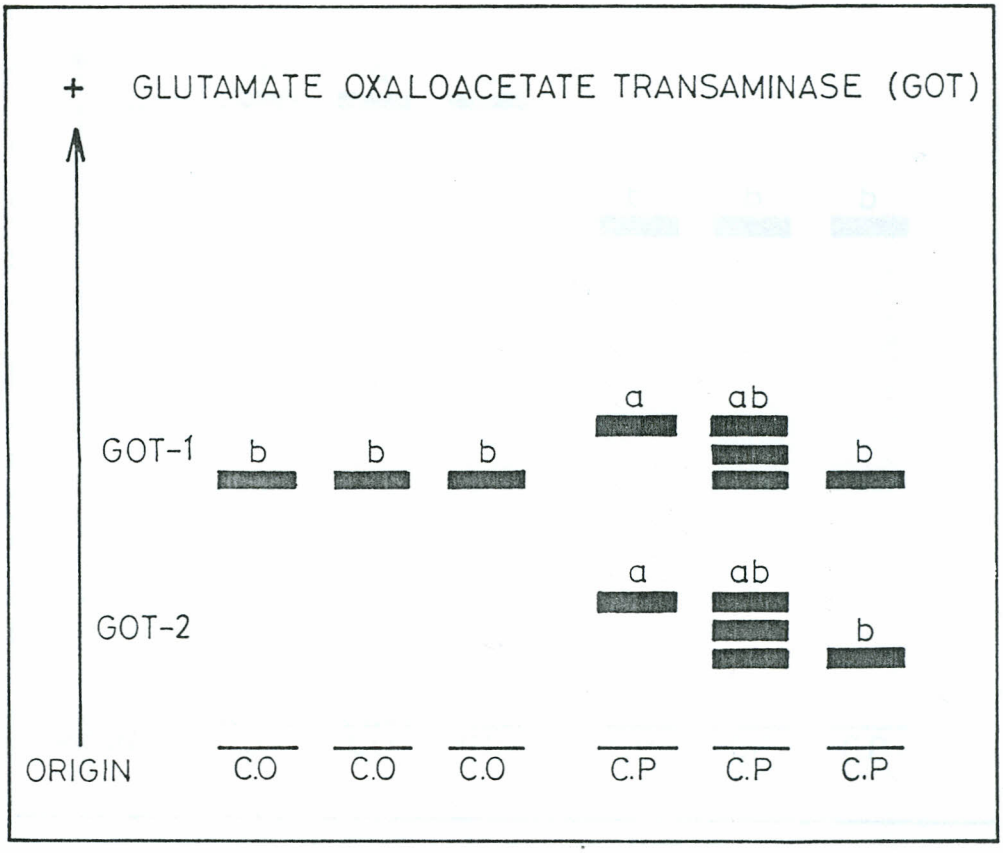

Fig.12: Glutamate oxaloacetate transaminase band patterns for C.orichalcociliellus (C.O) and C.partellus (C.P). (+) denotes the anodal end of the gel.

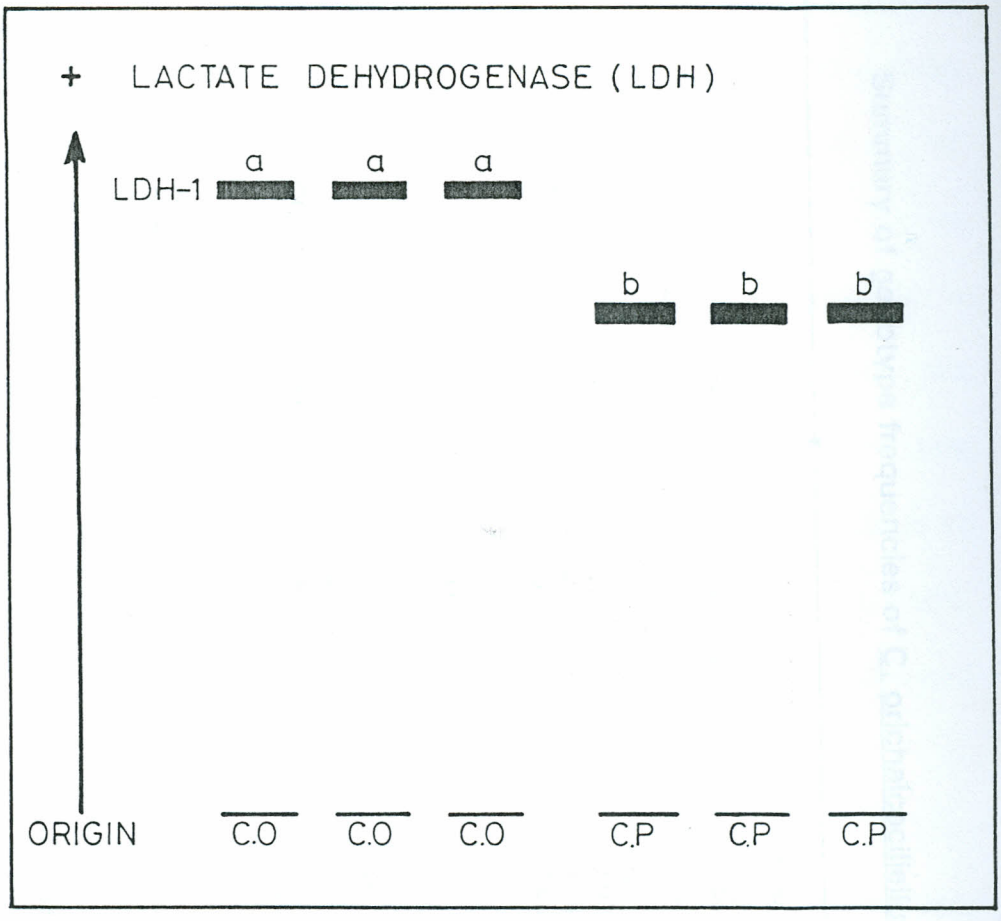

Fig.13: Lactate dehydrogenase (LDH) band patterns for C. orichalcociliellus (C.O) and C. partellus (C.P). (+) denotes the anodal end of the gel.

Table 9. Summary of genotype frequencies of C. orichalcociliellus and C. partellus larvae at four enzyme loci.

Enzyme		Locus	Band	Genotype	Chilo orichalcociliellus		Chilo partellus	
					Laboratory	Field	Laboratory	Field
GPI	N*				70	34	44	76
			a	aa	0.03	-	-	-
		GPI-1	ab	ab	0.14	0.20	-	-
			b	bb	0.83	0.80	-	-
			c	cc	-	-	0.89	0.92
			cd	cd	-	-	0.09	0.08
			d	dd	-	-	0.02	-
GOT	N				46	41	22	128
			a	aa	-	-	-	-
		GOT-1	ab	ab	-	-	0.18	0.03
			b	bb	1.00	1.00	0.82	0.97
			a	aa	-	-	0.95	0.73
		GOT-2	ab	ab	-	-	0.05	0.23
			b	bb	-	-	-	0.04
LDH	N				104	45	45	123
		LDH-1	a	aa	1.00	1.00	-	-
			b	bb	-	-	1.00	1.00

N = sample size

7:4 Discussion

The three enzymes GPI, LDH and GOT gave diagnostic bands for separating all *C. partellus* and *C.orichalcociliellus* larvae assayed. Perhaps the most successful application of electrophoresis has been in contributing to the precision of taxonomy (Grassel and Grassel, 1976; Miles, 1978; Britton-Davidan and Amourex, 1982). Many workers have used electrophoresis to solve taxonomic problems (Daly, 1989; Daly and Greg, 1985; Hassane, 1990; Pashley *et al.*, 1990; Rogo *et al.*, 1988). In the present study all the larvae assayed for GPI, LDH and GOT were successfully grouped either as *C. partellus* and or *C. orichalcociliellus*.

The use of electrophoresis for characterization of the very young larvae has certain pitfalls. Ideally, only one larvae should be homogenized for electrophoresis, but in practice, these small larvae presented problems. In quite a number of cases they did not stain and this may have been due to a low enzyme content. When several young larvae were homogenized together, visible bands were obtained. With field materials, valid comparisons may

not be possible when many larvae are homogenized together. In such a case more than two cotton threads should be used when loading so as to increase the amount of enzyme loaded.

The results obtained from the enzyme analysis agreed with the morphological identifications in all the cases where identification was first made based on the tubercles. The composition of the field collected larvae assayed was also in agreement with the recent observations (Overholt *et al.*, 1993) that *C. partellus* is more abundant than *C. orichalcociliellus*. Daly (1989) advocated that electrophoretic data must be supplemented by independent observations of the species' biology. For example, a complex of morphologically similar polychaeta annelids (Family: Capitellidae) was shown by Grassel and Grassel (1976) to be a species complex of six different species. This was demonstrated on the grounds of enzyme differences and confirmed by life-history traits. In the current study the distinctive asetose tubercles and the developmental study results confirmed the distinction of the two *Chilo* species obtained in the electrophoresis.

CHAPTER EIGHT

8 Summary And Suggestions For Future Research Work.

1. Comparative studies on the larval development period and number of instars were conducted in the laboratory under controlled conditions. Larvae of *C. partellus* completed five or six instars while those of *C. orichalcociliellus* completed six, seven or eight instars before pupation. The mean larval developmental period for *C. partellus* larvae was 24.9 days as compared to 34.3 days in *C. orichalcociliellus*.

2. The findings obtained from the comparative developmental studies demonstrated the existence of biological differences in *C. partellus* and *C. orichalcociliellus* larvae. The shorter developmental period for *C. partellus* gave it a superior competitive advantage over the indigenous *C. orichalcociliellus*. Future investigations should include studies on comparisons of larval developmental periods and determining the number of instars for the two species when reared on their natural hosts such as maize, sorghum and wild grasses.

3. The study assessed the use of morphological and biochemical characters in distinguishing between the larvae of *C. partellus* and *C. orichalcociliellus*. Morphological analyses indicated that diagnostic morphological characters were absent at some developmental stages and were thus not suitable as sole taxonomic tools in the distinction of the larvae of the two stemborer species studied.

4. Hexane soluble cuticular components were analysed using gas chromatography. There were no qualitative differences in the peak patterns of the two species. Quantitative differences in the peak percentage area indicated that the larvae from the laboratory had similar quantities of cuticular components irrespective of species. The same was observed for the field specimens. Cuticular component analysis as applied in this study could not therefore be considered a reliable taxonomic tool for separating *C. partellus* and *C. orichalcociliellus* larvae.

5. Analysis of enzyme systems on thin starch gel electrophoresis gave clear and consistent diagnostic bands for three enzyme systems. The migration of bands in the three enzyme systems, GPI, GOT and LDH, were completely

discriminatory between the two species. Enzyme analysis showed potential as a taxonomic tool for *Chilo* species in these studies. It became evident that integration of morphological and biochemical characters could form bases for the entire revision of larvae in the genus *Chilo.*

6. Further research in the cuticular component analysis should involve the isolation and identification of these cuticular components from the insects. Components should also be isolated from natural and artificial diet and their compositions compared with those of the insects to find out if there is a strong similarity in their chemistry.

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APPENDIX 1

Staining Recipes for the thirteen enzyme systems assayed.

1 Alpha-Glycerophosphate dehydrogenase

Tris A buffer(Tris-HCl, 0.3M, pH 7.4)	20 ml
α-D,L-Glycerophosphate	100mg
NAD (1% in water)	1ml
NBT (1% in water)	1ml
PMS (1% in water)	0.5ml
Incubate in the dark for about 30 min.	

2 Glutamate oxaloacetate transaminase

Tris A buffer	20 ml
L-Aspartic acid	200 mg
Ketoglutaric acid	100 mg
Pyridoxal-5-phosphate	10 mg
Dissolve and add Tris 1M to at least pH 7.4	
Incubate gel for 30 min. at room temp.	
Then add Fast Blue 100 mg	

3 Hexokinase

Tris A buffer	10 ml
ATP	20 mg
Alpha-D-glucose	500 mg
$MgCl_2$	1 ml
NAD	1 ml
NADP	0.5 ml
MTT	1 ml
Glucose-6-PDH	6 μl
PMS	0.5 ml

4 Glucose-6-phosphate dehydrogenase

Tris A buffer	10 ml
Glucose-6-phosphate	20 mg
NADP	1 ml
NBT	1 ml
PMS	0.5 ml

5 Malic enzyme

Tris A buffer	3 ml
Distilled water	3 ml
Malic acid	0.5 ml
NADP	0.5 ml
$MgCl_2$	1.0 ml
MTT	0.5 ml
PMS	0.5 ml

6 Isocitric dehydrogenase

Isocitric acid	30 mg
NADP	0.5 ml
$MgCl_2$	2.0 ml
MTT	0.5 ml
PMS	0.5 ml
Tris A buffer	3 ml
Distilled water	3 ml

7 Malate dehydrogenase

Malic acid	3 ml
Tris A buffer	3 ml
Distilled water	3 ml
NAD	0.5 ml
$MgCl_2$	1 ml
MTT	0.5 ml
PMS	0.5 ml

8 Alcohol dehydrogenase

Tris A buffer	6 ml
$MgCl_2$	0.5 ml
Ethanol	1 ml
NAD	0.5 ml
NBT	0.5 ml
MTT	0.5 ml
PMS	0.5 ml

9 Lactate dehydrogenase

Tris A buffer	3 ml

Lactic acid	3 ml
NAD	0.5 ml
NBT	0.5 ml
PMS	0.5 ml

10 Phosphogluco mutase

Tris A buffer	3 ml
Distilled water	3 ml
G6PD (100mg/ml)	20 μl
G-I-P (20mg/ml)	10 mg
$MgCl_2$	1 ml
NADP	0.5 ml
MTT	0.5 ml
PMS	0.5 ml

11 Glucose phosphate isomerase

Tris A buffer	3 ml
Distilled water	3 ml
Fructose-6-phosphate	10 mg
$MgCl_2$	2 ml
NADP	0.5 ml
PMS	0.5 ml
MTT	0.5 ml
G6PD	10 μl

12 Aldolase

Tris A buffer	25 ml
Fructose-1,6-diphosphate	0.1 g
NAD	2 ml
Sodium arsenate	0.06 g
G-3-PDH	50 μl
MTT	0.75 ml
PMS	0.25 ml

13 Esterase

Sodium phosphate buffer	15ml
α-Naphylacetate (0.3% in acetone)	0.5ml
β-Naphylacetate (0.3% in acetone)	0.5ml

Incubate with gel for 15-20 minutes
Add 15mg GBC salt shake until bands appear