UNIVERSITY OF
KWAZULU-NATAL

Sugarcane Stem Borers in Ethiopia: Ecology and

Phylogeography

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A Thesis submitted to the School of Biological and Conservation Sciences in

partial fulfilment of a
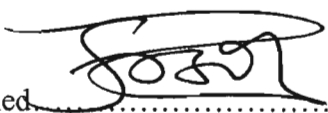
PhD Degree in Entomology

University of KwaZulu-Natal

Pietermaritzburg

South Africa

2006

DECLARATION

This thesis is the result of the author's original work except where acknowledged. I hereby certify that this research work has never been submitted for any degree or examination at any University or similar academic institution.

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Dr. Desmond Edward Conlong (Supervisor)

ACKNOWLEDGEMENTS

LIST OF PAPERS RESULTING FROM THIS THESIS THAT HAVE BEEN SUBMITTED TO OR ACCEPTED BY PEER REVIEWED JOURNALS

Assefa Y., Conlong D.E., Mitchell A. 2005. Mitochondrial DNA variation among populations of sugarcane borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae). *Proceedings of the South African Sugarcane Technologists' Association* 79: 382-385.

Assefa Y., Conlong D.E., Mitchell A. 2006. First record of the stem borer complex (Lepidoptera: Noctuidae; Crambidae; Pyralidae) in sugarcane estates of Ethiopia, their host plants and natural enemies. *Proceedings of the South African Sugarcane Technologists' Association* 80: 202-213.

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ABSTRACT

Eldana saccharina Walker (Lepidoptera: Pyralidae) is an indigenous insect widely distributed throughout Sub-Saharan Africa that is a major pest of sugarcane in southern Africa. Studies have shown that populations from West Africa have distinct behavioural differences compared to populations from eastern and southern Africa. In addition, the parasitoid guilds attacking populations in these regions are markedly different. This marked behavioural and parasitoid guild variation evoked a hypothesis of genetic diversification. To evaluate this hypothesis a project on the phylogeography of *E. saccharina* was initiated. The project was planned to include sampling of as many regions as possible in its known range in Africa, to obtain specimens of *E. saccharina* for genetic analysis.

When these surveys were initiated in Ethiopia, it was found that there was no published literature available on the occurrence of stem borers in Ethiopian sugarcane. It was thus clear that no stalk borer/parasitoid surveys had been completed in either sugarcane or any large grass and/ sedge indigenous hosts in Ethiopia. The study was thus expanded beyond the investigation of only the genetic diversity of *E. saccharina*, to include area-wide surveys to determine ecological aspects of the borer complex in suspected host plants, including sugarcane, in Ethiopia. In this way the host plant range and distribution of *E. saccharina* and other sugarcane borers in Ethiopia in particular could be determined, samples for a larger phylogeography project could be collected, and the insect's impact on sugarcane could be assessed.

Quantified area-wide surveys of the sugarcane estates and small-scale farmer fields of Ethiopia were conducted between December 2003 and February 2004. The surveys verified the presence of four lepidopteran stem borer species on Ethiopian sugarcane. These were *Chilo partellus* Swinhoe (Lepidoptera: Crambidae), *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae), *Busseola fusca* Fuller (Lepidoptera: Noctuidae) and *Busseola phaia* Bowden (Lepidoptera: Noctuidae). The surveys indicated that *Busseola* species are the major and most widely distributed sugarcane stem borers in sugarcane farms of Ethiopia. Over 70% of the peasant sugarcane fields visited were infested by these borers, with the highest levels of infestation (35% and 50%) being in the northern and western part of the country, respectively. *Busseola* was also the predominant stem borer of sugarcane in two of the three estates (Wonji and Finchawa). *Chilo partellus* and *S. calamistis* were recovered in very low numbers at all the commercial estates and from peasant farms in the western part of Ethiopia. However, *C. partellus* was the predominant sugarcane stem borer in lowland areas of northern, southern and eastern parts of the country. *Eldana saccharina* was recovered from large sedges in waterways of Metehara and Wonji sugar estates in the central part of the country, and sedges growing around lakes in northern and southern Ethiopia, but not from sugarcane anywhere in Ethiopia.

The phylogeographic study conducted on *E. saccharina* populations from eleven countries of Africa clearly showed the population structure of the insect within the continent. Five hundred and two base pairs of the mitochondrial DNA (mtDNA), corresponding to the Cytochrome Oxidase subunit I (COI) region, were sequenced to clarify phylogenetic relationships between geographically distant populations from eastern, northern, southern and western Africa. Results revealed that *E. saccharina* is separated into four major populations corresponding to their geographical location, i.e. West African, Rift Valley

and two southern African populations. Sequence divergence between the four populations ranged from 1% to 4.98%. The molecular data are congruent with an isolation by distance pattern except for some of the specimens from eastern and southern Africa where geographically close populations are genetically distant from each other. Geographical features such as the Rift Valley and large water bodies in the continent seem to have a considerable impact on the genetic diversity in *E. saccharina*.

Identification of field-collected stem borer specimens was done using classical taxonomic techniques, except for *Busseola* spp. where DNA barcoding was used. As field-collected larval material of *Busseola* died before reaching the adult stage, identification of species using adult morphology was not possible. Sequence divergence in the COI gene was used as a tool to identify the species of *Busseola* attacking Ethiopian sugarcane. Partial COI sequences from Ethiopian specimens were compared with sequences of already identified noctuid species from the East African region. Results of the sequence analysis indicated that the *Busseola* species complex in Ethiopian sugarcane comprised *B. fusca* and *B. phaia*. Sequence divergences between Ethiopian *Busseola* species was as high as 5.0 %, whereas divergences within species were less than 1% in both species identified.

Several larval parasitoids, bacterial and fungal diseases of stem boring caterpillars were also recorded in Ethiopian sugarcane. Amongst these was *Cotesia flavipes* Cameron (Hymenoptera: Braconidae). This exotic parasitoid has been introduced into several African countries for the control of *C. partellus* in maize and sorghum, but had never been released in Ethiopia. To investigate the origin of *C. flavipes* in Ethiopian sugarcane, molecular analyses were conducted on Ethiopian specimens from sugarcane and specimens of *C. flavipes* from different countries of Africa released from the Kenyan laboratory

colony, again using COI sequences. Results of the analysis revealed that the *C. flavipes* population that had established in sugarcane fields of Ethiopia was similar to the south east Asian populations released against *C. partellus* in maize in other parts of Africa, and different from other populations of this species, providing evidence that the Ethiopian *C. flavipes* is likely to be a descendant of the original Pakistani population that was released in different parts of Africa.

The study reveals the importance of lepidopteran stem borers in sugarcane production in Ethiopia and highlights the role of molecular methods in species identification and determining phylogenetic relationships. More importantly, this study establishes the continental phylogeographical pattern of the indigenous moth, *E. saccharina*. The impact of geological events, geographic barriers and cropping systems on the evolution, distribution and abundance of stem borers are discussed. Future areas of research for understanding more about the phylogeographic relationships of *E. saccharina* and management of stem borers are discussed.

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CHAPTER 1

GENERAL INTRODUCTION

1.1   BACKGROUND

Sugarcane, *Saccharum* spp. L. (Poaceae) is a perennial crop that is grown as a source of sugar primarily in the tropical and subtropical areas of the world, including several countries in Africa, the Mascarene Islands and Madagascar (Overholt *et al.* 2003). The taxonomic status and the origins of cultivated sugarcane varieties are not clear but the varieties of noble canes, *Saccharum officinarum* L., are thought to have originated in Melanesia and the ancestral form is thought to be the wild *Saccharum robustum* L. of New Guinea and adjacent islands (Pemberton and Williams 1969). Other cultivated sugarcanes, *Saccharum barberi* L. and *Saccharum sinense* L., are believed to have been derived through natural hybridization of *S. officinarum* with the wild *Saccharum spontaneum* L. (Stevenson 1965). Sugarcane has been grown in gardens in New Guinea since time immemorial (Pemberton and Williams 1969) and cultivation of the crop in Africa and neighbouring islands was first recorded in the Cape Verde Islands in the early 15th century (Polaszek and Khan 1998).

Currently, sugarcane is a cash crop providing a major source of income for many countries around the world. In 2004, a total of 20 114 934 hectares was allocated to sugarcane in 100 countries of the World (FAO 2004). These occur in eight geographical areas: Africa and the Mascarene islands; Southeast Asia; India and Pakistan; Australia; Indonesia and the Pacific islands; South America; Central America and the Caribbean islands; and North

America (Conlong 1994; Carnegie and Conlong 1994). Africa plays only a minor role in world sugarcane production with an output of less than 10% of the total (Lichtss 2002). Out of a total of 148 845 000 tonnes of sugar produced worldwide in 2002/03, sugarcane producing countries of Africa produced only 9 931 000 tonnes of sugar (FAO 2004). A large quantity of this sugar was produced by South Africa and Egypt followed by Mauritius, Sudan, Zimbabwe and Kenya (Tarimo and Takamura 1998). The relatively small quantity of sugar produced in Africa, however, plays a very significant role, for its production is labour intensive and vital to the economy of African nations (FAO 2004). In Africa's leading sugarcane producer, South Africa, sugarcane production is a source of employment for 350 000 people and there are more than 50 000 registered cane growers, more than 47 000 of which are small-scale growers (SASA 2006). Ethiopia stands 16th in Africa in terms of areas of land allocated for sugarcane, and its sugarcane production was ranked 9th in 2003 (FAO 2004). In Ethiopia, sugarcane is grown mainly by subsistence or peasant farmers for the chewing market, and is a source of income to more than 500 000 of them (EASE 2003). Many employees in the Ethiopian sugar estates also depend on sugarcane production for their livelihood.

A great variety of insect pests feed on sugarcane, including insects from a broad spectrum of orders such as Lepidoptera, Coleoptera, Hemiptera, Orthoptera and Isoptera (Pemberton and Williams 1969; Conlong 1994; Carnegie and Conlong 1994; Leslie 2004). Wade (1951) listed 1 277 species of insects associated with sugarcane worldwide. However, only a limited number are of economic importance.

Stem borers are amongst the most destructive pests of sugarcane. They attack the crop at all stages of its growth and all parts of the stalks. Borers such as *Eldana saccharina*

Walker (Lepidoptera: Pyralidae) attack the lower portions of mature canes (Conlong 2001), whereas *Chilo agamemnon* Bles. (Lepidoptera: Crambidae) prefers the shoots of young sugarcane. *Diatraea saccharalis* F. (Lepidoptera: Crambidae) attacks the top of stalks resulting in dead hearts (Leslie 2004). The attack on the upper portions of the stalk decreases sugarcane biomass, while damage to the lower portion of the stalks leads to a marked reduction in sucrose. The loss in sucrose content and quality is further aggravated by the actions of fungal pathogens that produce a red discoloration in the bored stalks (Leslie 2004).

1.2 ABUNDANCE, DISTRIBUTION AND ECONOMIC IMPORTANCE OF SUGARCANE STEM BORERS

Several species of stem borers are reported to be associated with sugarcane where this crop is cultivated (Leslie 2004). Most of these belong to the order Lepidoptera, although coleopteran and dipteran borers have also been recorded (Conlong 1994). Bleszynski (1969) listed 33 lepidopteran stem borers of sugarcane, which include 21 species in genus *Diatraea* (Lepidoptera: Crambidae) and seven in *Chilo* (Lepidoptera: Crambidae). Rao and Nagaraja (1969) listed 11 species of *Sesamia* (Lepidoptera: Noctuidae) attacking sugarcane in different parts of the world of which four, *Sesamia calamistis* Hampson, *Sesamia inferens* Walker, *Sesamia grisescens* Walker and *Sesamia uniformis* Dudgeon were serious pests of sugarcane. Of 15 lepidopteran stem borers that are associated with sugarcane in Africa and the surrounding islands only five were considered to be of economic importance (Leslie 1994). While in Taiwan, five species of moth borers have been found attacking sugarcane (Cheng 1994). Kuniata (1994) specified two coleopteran and 11 lepidopteran stem borers associated with Papua New Guinean and Indonesian sugarcane.

Only few of the stem borers (11 to 20 species) recorded from world sugarcane were regarded as significant pests (Conlong 1994; Leslie 2004).

The geographic distribution of most of these insect pests is very narrow and each geographic region has a distinctive sugarcane pest fauna (Pemberton and Williams 1969). It is generally difficult to relate the distribution of sugarcane insect pests to the origins and distribution of the cultivated sugarcane species. For instance all the sugarcane insects native to the New World are indigenous to regions outside the natural range of wild *Saccharum* species. Stem borer species in the genus *Diatraea*, are still restricted to the New World and almost all the stem borers in the genus *Chilo* are restricted to the Old World despite the world-wide cultivation of sugarcane (Bleszynski 1969; Leslie 2004). Pemberton and Williams (1969) related the association of insect pests with sugarcane to adaptation of the insects to the plant consequent to its extensive cultivation. The African sugarcane borer, *Eldana saccharina*, and the Mexican rice borer, *Eoreuma loftini* Dyar (Lepidoptera: Crambidae) from Central and South America are amongst the pests that adapted to feed on sugarcane as a result of cultivation of the crop in areas previously occupied by their natural wild host plants (Conlong 1994). However, the distribution of these insects has often been extended by accidental introduction of species from one country to another. Therefore, the pest fauna of any region comprises a mixture of indigenous and alien species. The accidental introduction of the lepidopteran stem borer, *Chilo sacchariphagus* (Bojer) (Lepidoptera: Crambidae) into mainland Africa (Way and Turner 1999) is one example of such expansion by insect pests of sugarcane. However, the sugarcane pest fauna in mainland Africa is dominated by indigenous species. Of the five major stem borers of sugarcane reported by Leslie (2004) from mainland Africa, only one species (*C. sacchariphagus*) is exotic to the continent.

In contrast to this, alien species dominate the sugarcane insect faunas in oceanic islands, as the insular faunas on the islands provide few pests. Ganeshan (2001) reported over forty species of insects feeding on sugarcane in Mauritius; nearly all of them being foreign species accidentally introduced to the island. Generally, pest faunas of sugarcane are of dual origin with indigenous pests dominating in the continents and exotic pests being dominant in the islands (Pemberton and Williams 1969).

It is usually difficult to estimate the loss caused by borers. Different researchers have developed different methods to estimate the amount of sucrose lost because of borers. In his study on the management of *S. grisescens* in Papua New Guinea, Kuniata (1994) reported an 18% loss in sugarcane production valued at approximately 4.2 million US dollars. Similarly, the average annual yield loss in South African sugarcane due to *E. saccharina* for the period 1980/81-1985/86 was estimated to be R60 million (10 million US dollars) (Kasl *et al*. 2003). In Mauritius, *C. sacchariphagus* causes an average loss of 0.8% recoverable sucrose for every 1% of internodes bored (Ganeshan 2001). In Indonesia, the yield loss due to *C. sacchariphagus* was reported to reach 10% of recoverable sugar for 20% internodes bored (Kuniata 1994). In Taiwan, up to 43% reduction in recoverable sucrose was recorded due to 8.9% level of infestation by stem borers (Cheng 1994).

1.3   IMPORTANT SUGARCANE STEM BORERS IN AFRICA

Sugarcane stem borers in Africa and neighboring islands are almost all lepidopteran, with most species belonging to the families Crambidae, Pyralidae and Noctuidae. Pests have been recorded from the genera *Chilo, Sesamia* and *Eldana* (Leslie 1994; Conlong 1994)

with the last genus only including one major pest species. Researchers have reported several lepidopteran stem borers of African sugarcane from various parts of the continent. These are summarized in Table 1.1. Only a few of these stem borers listed were reported to cause a significant damage to the crop.

Table 1.1   A list of stem borers associated with sugarcane in Africa and neighboring islands (?= information unavailable)

Species	Family	Distribution in Africa	Associated with sugarcane	References
Acigona leucomeralis?	Crambidae	?	?	Leslie (1994)
Angustalius malacelloides Bleszynski	Crambidae	?	?	Leslie (1994)
Bissetia poliella Hampson	Crambidae	?	Ethiopian region	Bleszynski (1969)
Chilo agamemnon Bleszynski	Crambidae	Egypt, Sudan and Uganda	Egypt	Maes (1998); Leslie (2004)
Chilo orichalcociliellus Strand	Crambidae	East Africa, southern Africa and Madagascar	Somalia	Maes (1998)
Chilo partellus Swinhoe	Crambidae	East and southern Africa	East Africa, southern Africa	Maes (1998); Bleszynski (1969); Way and Kfir (1997)

Species	Family	Distribution in Africa	Associated with sugarcane	References
Chilo sacchariphagus Bojer	Crambidae	Mozambique, Mascarene islands	Mozambique, Mascarene islands	Leslie (2004); Maes (1998); Conlong and Goebel (2002)
Chilo zacconius Bleszynski	Crambidae	West Africa	Uganda	Maes (1998); Leslie (1994); Ingram (1958)
Coniesta ignefusalis Hampson	Crambidae	Sub-Saharan Africa	Nigeria	Maes (1998); Harris (1962)
Metamasius hemipterus L.	Curculion-idea	?	West Africa?	Hill (1983)
Tetramoera schistaceana Snellen	Eucosmid-ae	Mascarene islands	Mascarene islands	Ganeshan (2001)
Busseola fusca Fuller	Noctuidae	Sub-Saharan Africa	Nigeria, Ivory Coast and Ethiopia	Holloway (1998) Conlong (2000); Assefa *et al.* (unpublished); Harris (1962)
Busseola phaia Bowden	Noctuidae	East Africa	Ethiopia	Nye (1960); Assefa *et al.* (unpublished)

Species	Family	Distribution in Africa	Associated with sugarcane	References
Busseola segeta Bowden	Noctuidae	?	Uganda	Ingram (1958)
Sesamia calamistis Hampson	Noctuidae	Tropical Africa, southern Africa and Mascarene islands	Most of Tropical Africa, southern Africa and Mascarene islands	Polaszek (1998); Rao and Nagaraja (1969); Ganeshan (2001)
Sesamia cretica Lederer	Noctuidae	North-East Africa	Egypt, Algeria, Morocco and Somalia	Holloway (1998); Rao and Nagaraja (1969)
Sesamia nonagrioides botanephaga Tams and Bowden	Noctuidae	Ghana, Togo, Ivory Coast, Sudan, Nigeria, Uganda and Ethiopia	Ghana, Nigeria, Uganda and Ethiopia	Rao and Nagaraja (1969); Getu *et al.* (2001); Tams and Bowden (1953)
Sesamia nonagrioides Lefebvre	Noctuidae	?	North Africa	Rao and Nagaraja (1969)
Sesamia penniseti Tams and Bowden	Noctuidae	western and Central Africa	Nigeria	Harris (1962); Holloway (1998)
Sesamia peophaga Tams and Bowden	Noctuidae	western and Central Africa	Nigeria and Uganda	Holloway (1998); Harris (1962); Ingram (1958)
Eldana saccharina Walker	Pyralidae	Sub-Saharan Africa	Sub-Saharan Africa	Leslie (2004); Kfir *et al.* (2002)

1.4   MOLECULAR STUDIES OF INDIGENOUS STEM BORERS AND THEIR NATURAL ENEMIES

The exceptional diversity of insects is well known, but its underlying causes have been the subject of considerable speculation (Baer *et al.* 2004). The genetic differentiation among insect populations may be a result of specialization on different host species (Mitter *et al.* 1988; Farrell 1998) or isolation due to geographic barriers (Sezonlin *et al.* 2005; Assefa *et al.* 2006) or both. Host specialization may result in genetic divergence of lineages that shift to novel hosts, leading ultimately to reproductive isolation and the formation of new species (Baer *et al.* 2004). Spatial variation in co-evolution can also lead to divergence in host use between populations or in different parts of the species' distribution (Conlong 2001, Assefa *et al.* 2006). For widely distributed indigenous African stem borers, which feed on various host plants, and for the natural enemies being used in stem borer management, the question of genetic diversity is not only pervasive but has important implications for their management (Conlong 2001; King *et al.* 2002; Sezonlin *et al.* 2005; Assefa *et al.* 2006).

The invention of PCR technology and the widespread use of DNA sequencing and multilocus markers have made studies on genetic differentiation relatively easy, fast and successful. Mitochondrial DNA (mtDNA) has proven powerful for genealogical and evolutionary studies of animal populations and it is the most commonly employed marker for determining genetic relationships among populations (Sperling *et al.* 1999; Avise, 2000; Scheffer 2000; Segrave and Pellmyr 2001; King *et al.* 2002; Simmons and Scheffer 2004). Due to maternal inheritance and a relatively fast rate of evolution, mitochondrial DNAs have been used to provide insights into population genetic structure, gene flow,

biogeography and intraspecific relationships (Moritz *et al.*1987; Danforth *et al.* 1998; Sperling *et al.* 1999; Simmons and Scheffer 2004). Such data are also capable of revealing cryptic lineages representing distinct species within geographically widespread and apparently morphologically homogeneous organisms (Scheffer 2000).

Mitochondrial DNA has been used to reconstruct the phylogenetic and phylogeographic relationships in indigenous stem borers (King *et al.* 2002; Sezonlin *et al.* 2005; Assefa *et al.* 2006) and their parasitoids (Dittrich *et al.* 2006; Muirhead *et al.* 2006). However, the number of taxa addressed, thus far are very few. In this study (chapter 3) the phylogeography of *E. saccharina* and phylogeny of *Busseola* species and *C. flavipes* are described. These studies are the first steps towards understanding the population structure of important insect pests and their natural enemies in Africa. Such approaches lead to a better understanding of the population structure of important insect pests, which in turn allows more sound and scientific insect management systems to be planned and implemented.

1.5   RESEARCH OBJECTIVES

1.5.1   General Objectives

The aim of this project was to determine the stem borer species assemblage in Ethiopian sugarcane, the species' host plant ranges and their parasitoid assemblages. In addition, because of the pan-African distribution and suspected existence of biotypes, the aim was to characterize the genotypic diversity of some of the insect species (such as *E. saccharina* and *C. flavipes*) from various parts of Africa and generate ecological information and molecular diagnostic methods that can be used for the development of sound biological

control programs for the management of these pest insects and the efficient use of their natural enemies.

1.5.2   Specific Objectives

Specifically the project is planned to:

- Examine the pattern of genetic variation among populations of *E. saccharina* from different geographic regions of Africa and to assess the utility of this information for planning future biological control programs

- Assess the distribution and the diversity of sugarcane stem borer species occurring in the peasant and estate farms of Ethiopia

- Evaluate the effect of Ethiopian farming systems on the abundance of stem borers and their natural enemies

- Investigate the existence of within and between species genetic diversity in the *Busseola* species complex in Ethiopian sugarcane

- Examine the genetic variation of *C. flavipes* reared from stem borers in Ethiopia in order to determine the origin of this exotic parasitoid in Ethiopian sugarcane.

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CHAPTER 2

ECOLOGY OF SUGARCANE STEM BORERS IN ETHIOPIA

2.1 IMPORTANT SUGARCANE STEM BORERS IN ETHIOPIA

The importance of stem borers in sugarcane production in Ethiopia has long been recognized. However, their species composition, distribution and relative importance in Ethiopian sugarcane fields has never been studied. There is no published information available on the complex of sugarcane stem borers existing in the country. Due to research priorities being focused on food crops, several countrywide surveys were conducted, and only stem borer species attacking cereal crops in Ethiopia were identified (Gebre-Amlak 1985; Getu *et al.* 2001). Of the diverse stem borers attacking sorghum and maize, only three species, *Chilo partellus* Swinehoe (Lepidoptera: Pyralidae), *Busseola fusca* Fuller (Lepidoptera: Noctuidae) and *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae), are known to be economically important (Gebre-Amlak 1985; Getu *et al.* 2001).

The status of these species in Ethiopian sugarcane is discussed in this Chapter. The Chapter also reveals that some indigenous noctuid species are expanding their host range to include sugarcane. *Busseola phaia* Bowden (Lepidoptera: Noctuidae) was recorded as a pest of sugarcane in the country for the first time. This insect has been reported to be common in wild host plants (Nye 1960), but has never been recorded as a pest in crop fields. Its presence in Ethiopian sugarcane fields suggests that other stem borers recorded in wild hosts may also be able to invade crops. This phenomenon already has been reported from Zimbabwe where *Eldana saccharina* Walker (Lepidoptera: Pyralidae) colonized

sugarcane by moving from its natural habitat in indigenous sedges (Mazodze *et al.* 1999; Mazodze and Conlong 2003).

Stem borer species recorded in the country are listed in Table 2.1 and life stages of the most important ones are shown in Figure 2.1.

Table 2.1 Lepidopteran stem borer species recorded from Ethiopia, their distribution and host plants

Species	Natural hosts	Crop host	Distribution	References
Busseola fusca Fuller	*Pennisetum purpureum* Schumach. (Poaceae), *Phragmites mauritianus* Kunth (Poaceae)	maize, sorghum, sugarcane	Sub-Saharan Africa	Holloway (1998); Conlong (2000); Harris (1962)
Busseola phaia Bowden	*Panicum maximum* L. (Poaceae), *Panicum deustum* Thunb. (Poaceae)	maize, sugarcane	Kenya, Uganda, Ethiopia, Cameroon	Nye (1960); Assefa *et al.* (unpublished)
Sesamia calamistis Hampson	*Cyperus articulatus* L. (Cyperaceae)	maize, sorghum, sugarcane	Tropical Africa	Polaszek (1998)
Sesamia nonagrioides Lefebvre	*Typha domingensis*, Pers. (Typhaceae), *P. purpureum*, *Echinochloa pyramidalis* (Lam.) (Poaceae)	maize, sorghum, sugarcane	Africa, Europe	Getu *et al.* (2001); Holloway (1998)
Sesamia	*P. maximum*	sugarcane	Eastern western	Holloway (1998);

peophaga Tams and Bowden			and southern Africa	Harris (1962); Ingram (1958); (Le Rü[1] personal communication)
Sciomesa mesophaea Hampson	*T. domingensis*	Not reported	Eritrea, Ethiopia, Kenya, Tanzania	(Le Rü[1] personal communication)
Sciomesa piscator Hampson	*P. purpureum, Arundo donax* L. (Poaceae), *Cenchrus ciliaris* L. (Poaceae), *Chloris gayana* Kunth. (Poaceae), *E. pyramidalis, Eriochloa meyeriana* (Nees) (Poaceae*)*, *P. cladestinum, P. macrourum, P. mauritianus, Setaria sphacelata* Schumach. (Poaceae), *C. atroviridis, C. dives, C. latifolius, C. involucratus, C. rotundus, C. papyrus* L. (Cyperaceae)	Not reported	Eastern and southern Africa	(Le Rü[1] personal communication)
Sciomesa jemjemensis Laporte	*Setaria megaphylla* (Steud.) (Poaceae)	Not reported	Ethiopia	(Le Rü[1] personal communication)

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[1]Dr Bruno Le Rü, Institut de Recherche pour le Dévelopment(IRD)/ ICIPE, Nairobi, Kenya.

Species	Natural hosts	Crop host	Distribution	References
Manga nubifera Hampson	*P. maximum*	Not reported	Eastern and southern Africa	(Le Rü[1] personal communication)
Sesamia cretica Lederer	*Sorghum halepense* (L.) Pers. (Poaceae)	sorghum, maize	North and East Africa	Holloway (1998); Rao and Nagaraja (1969)
Chilo partellus Swinhoe	*S. halepense, S. verticilliflorum, P. maximum, P. purpureum*	sorghum, maize, sugarcane	East and southern Africa	Maes (1998); Way and Kfir (1997); Gebre-Amlak (1985)
Eldana saccharina Walker	*C. papyrus, C. dives*	sorghum, maize, sugarcane	Sub-saharan Africa	Conlong (2001); Assefa *et al.* (2006)
Tortricidae sp.	*C. articulatus, Scirpus inclinatus*	Not reported		(Le Rü[1] personal communication)
Ematheudes spp.	*P maximum*	Not reported		(Le Rü[1]personal communication)

Busseola fusca (M)   *Busseola fusca* (F)   *Busseola fusca* (L)

Busseola phaia (M)   *Busseola phaia* (F)   *Busseola phaia* (L)

Chilo partellus (M)   *Chilo partellus* (F)   *Chilo partellus* (L)

Not available

Eldana saccharina (M)   *Eldana saccharina* (L)
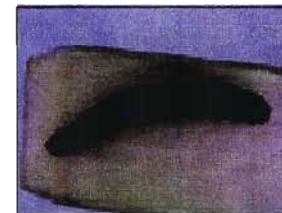
Not available

Ematheudes sp.1 (M)   *Ematheudes sp.1* (F)

Ematheudes sp.2 (M)   *Ematheudes sp.2* (F)   *Ematheudes sp.2* (L)
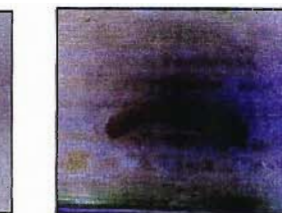
Manga rubifera (M)
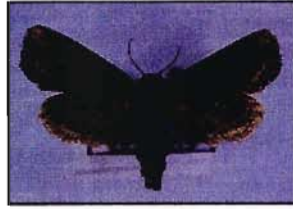
Manga rubifera (F)
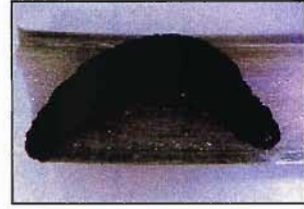
Manga rubifera (L)

Sciomesa jemjemensis (M)

Sciomesa jemjemensis (F)

Sciomesa jemjemensis (L)

Sciomesa piscator (M)

Sciomesa piscator (F)
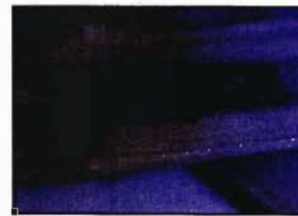
Sciomesa piscator (L)
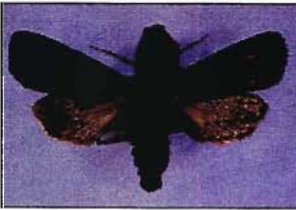
Sciomesa mesophaea (M)

Sciomesa mesophaea (F)

Sciomesa mesophaea (L)

Sesamia calamistis (M)

Sesamia calamistis (F)

Sesamia calamistis (L)

Sesamia cretica (M)

Sesamia cretica (F)

Sesamia cretica (L)

Sesamia nonagrioides (M)   Sesamia nonagrioides (F)   Sesamia nonagrioides (L)

Sesamia poephaga (M)   Sesamia poephaga (F)   Sesamia poephagas (L)

Not available

Tortricidae (M)   Tortricidae (L)
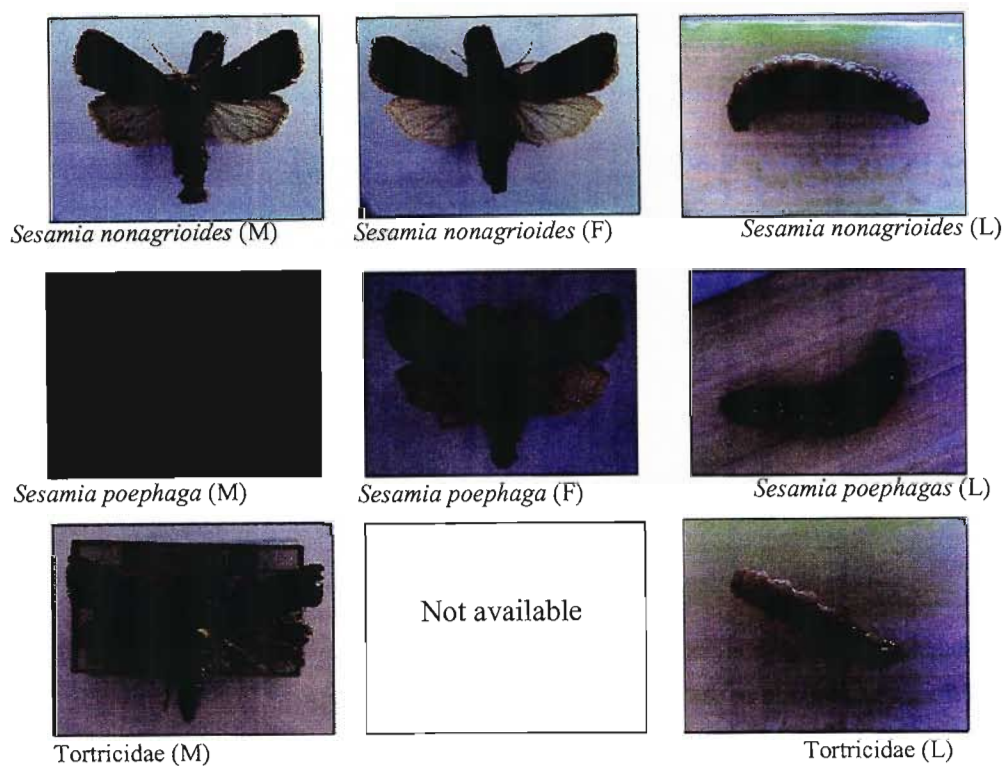
Figure 2.1   Photographic examples of adults and larvae of the lepidopteran stem borers known to occur in Ethiopia (From Le Rü, personal photographs). M=male, F=female, L=larva

More than one third of the land under sugarcane in Ethiopia is owned by peasant farmers who grow sugarcane in their garden and/or in the field under irrigation. In these farms, sugarcane is planted near or mixed with other crops such as sorghum, maize, coffee, fruit and vegetables. The majority of the sugarcane plantations, however, belong to three government owned commercial farms (Finchawa, Wonji and Metehara). The differences in cropping practices between the monocultures of the big estates and the multiple cropping of the peasant farms have been reported to have different effects on pests (Ingram 1958; Lawani 1982; Gebre-Amlak 1988; Päts 1996).

The total stem borer complex found in surveys in 45 peasant farmers' fields are summarized in Table 2.2 and the distribution of the stem borer species recorded in Ethiopia is shown in Figure 2.2. Locality names, altitudes and geographic coordinates of the surveyed peasant sugarcane plots are indicated in the sections specific to the particular stem borer species, together with details on the status of each stem borer species recorded. Sugarcane stem borer species in the estate farms of Ethiopia (Chapter 2.3) and the status of *E. saccharina* (Chapter 2.6) in the country are discussed separately to clearly show the differences in stem borer complex between estate farms and peasant sugarcane plots, and the importance of indigenous hosts as refuges of stem borers in sugarcane agroecosystem.

Table 2.2 The total stem borer complex found during surveys of peasant farms in the major sugarcane producing regions of Ethiopia.

Region	Altitudinal Range (Meters above sea level)	No. of peasant farms visited	Status of stem borers in the peasant farms			
			Busseola spp	*C. partellus*	*E. saccharina*	*S. calamistis*
Oromia	1410-2060	20	15	5	0	0
Amhara	1310-1930	17	9	8	0	0
SNNPR	1640-1880	8	8	0	0	0
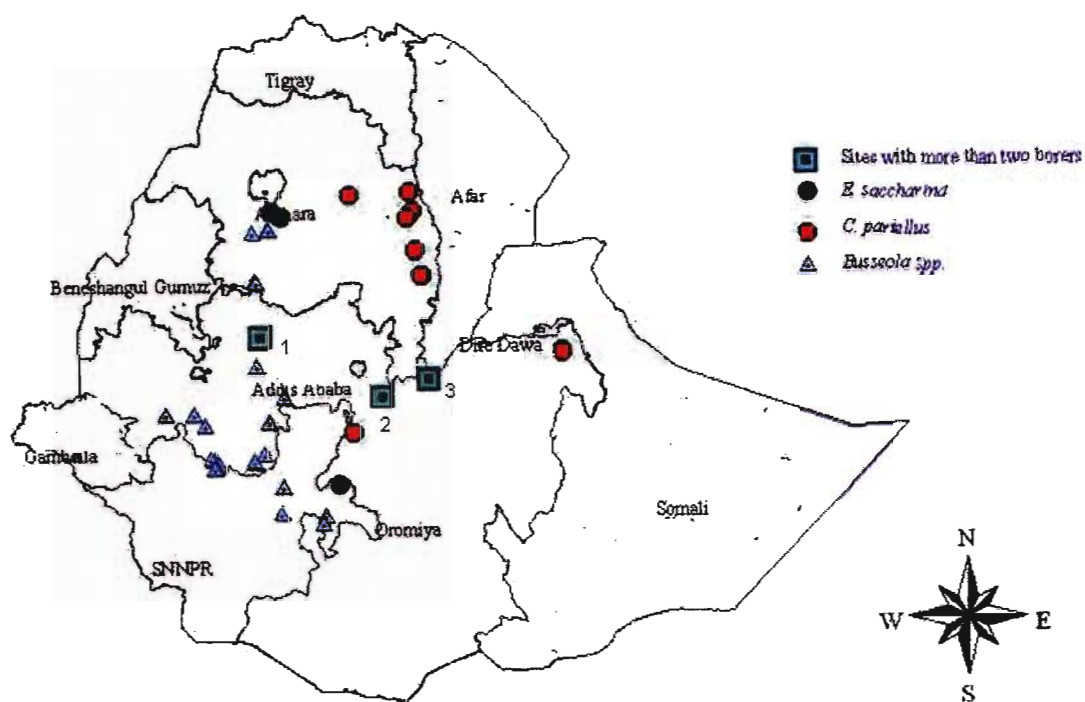
Figure 2.2 Map of Ethiopia showing the distribution of stem borers recorded in this study. (1= *Busseola* spp., *Chilo partellus* and *Sesamia calamistis*, 2= *Busseola* spp., *C. partellus*, *S. calamistis* and *Eldana saccharina*, 3= *Busseola* spp., *S. calamistis* and *E. saccharina*). The locality names, geographic locations and the status of the stem borers are indicated in the respective species specific sections of this chapter.

The following sections in this Chapter deal with the complex of noctuids, crambids and pyralids attacking sugarcane in the commercial estates (2.3), the impact on sugarcane, indigenous host plants and natural enemies of the *Busseola* spp. recorded on peasant farms (2.4), *Chilo partellus* on peasant farms (2.5) and *E. saccharina* in Ethiopia (2.6).

2.2 GENERAL DESCRIPTION OF THE STUDY AREAS AND SURVEY METHODS

2.2.1 Study areas

2.2.1.1 Sugarcane estate farms

The commercial sugarcane estates visited are Finchawa (09°52′N; 37°19′E), Metehara (08°49′N; 39°58′E) and Wonji (08°31′N; 39°12′E). These are government owned farms that have their own mills and are the sole suppliers of sugar for local consumption and the export market. They are located in lowland areas, in the maize and sorghum belts of the country. Mean daily temperatures in the estates range from 20.8 to 24.5°C and they receive an annual rainfall of 545 to 1208 mm. These estates are government owned farms that grow sugarcane commercially in an area more than 24000 ha in extent. Sugarcane on the estates is irrigated from two large perennial rivers (Awash and Finchawa). Currently, the estates plant more than 10 different varieties of sugarcane imported from different countries of the world.

2.2.1.2 Peasant sugarcane plots

The surveys on peasant farms covered 45 peasant farmers' plots in the three major sugarcane-growing regional states of Ethiopia: Oromia, Amhara and SNNPR. The sites visited are situated in mid altitude, 1500-2000 meters above sea level (m.a.s.l.) and lowland areas (below 1500 m.a.s.l.) and receive an annual rainfall ranging from 950mm to 1500mm (Habtu *et al.* 1996).

The small-scale farms are peasant plots as small as 0.01 ha, and rarely exceed 0.5 ha in size. Peasants grow sugarcane in their gardens under rain-fed conditions, but some irrigate when fields are close to water sources. In the small-scale farms, sugarcane is planted near or mixed with other crops such as sorghum, coffee, maize, fruit and vegetables. The sugarcane produced by these farmers is sold to suburban settlers for chewing and rarely to traders. None of the produce is supplied to mills for sugar production. Approximately 5144, 1013 and 6679 ha of land is allocated for small-scale sugarcane production in Oromia, Amhara and SNNPR regions, respectively (EASE, 2003).

2.2.2   Survey methods

Surveys were conducted during December 2003 to February 2004 and again from November to December 2004. During these, sugarcane of different ages, as well as indigenous host plants (large grasses and sedges) in wetlands, irrigation canals and field borders were examined for possible infestation by stem borers and the presence of any natural enemies.

2.2.2.1 Sampling in sugarcane estate farms

Plots to be evaluated were randomly identified from production data sheets from each estate, with due consideration given to include all varieties and age groups. Selected sugarcane plots and indigenous host plants growing in irrigation canals, swampy areas, reserve dams and field margins were inspected for signs of stem borer infestation, such as the presence of dead hearts, larval frass and/or adult exit holes. Levels of infestation were estimated from 100 randomly selected sugarcane and/or 25 to 100 wild host plants from different corners of the selected fields. In sugarcane fields, 30 of the 100 sample plants

were randomly inspected by walking from the top left corner diagonally to bottom right corner of the field and another 30 samples were from the top right corner diagonally to the bottom left corner. The remaining 40 samples were inspected by walking through the field from the center of each side of the field, 20 per side. Stalks were examined *in situ*. In surveys of border rows, wetlands and waterways nearby sugarcane fields, 25-100 indigenous grass host plant stems were collected and inspected for borers and their natural enemies, as described above for sugarcane. In *Cyperus* spp. the insect is known to bore into the umbels and rhizomes (Conlong 1990). Hence, the umbels and rhizomes not covered with water were inspected. In large indigenous grasses, attention was given to the stalks only.

2.2.2.2 Sampling in peasant sugarcane plots

Survey regions were identified from sugarcane production statistics (EASE 2003). Within these regions, survey sites were selected on the basis of the presence of known host plants and accessibility. In localities visited, fields of sugarcane and indigenous host plants were inspected for signs of stem borer infestation, such as the presence of larval frass and/or adult exit holes, and selected on the basis of presence of such infestation signs. After identification of infested fields, the geographic coordinates (altitude, latitude and longitude) were recorded using a GARMIN 12X portable Global Positioning System (GPS) and an altimeter. Levels of infestation were estimated from 25 to 100 randomly selected sugarcane and/or wild host plants from different corners of the selected fields. By counting the number of bored plants (indicated by the presence of frass, adult exit holes on the rind of the stalk and/or dead hearts), and relating that to the total number of plants examined per field, percentage stalk damage could be determined. Stalks were examined *in*

situ. For square and rectangular sugarcane fields, 30 of the 100 sample plants were randomly inspected by walking from the top left corner diagonally to the bottom right corner of the field and another 30 samples were from the top right corner diagonally to the bottom left corner. The remaining 40 samples were inspected by walking through the field from the center of each side of the field. This method and the number of plants inspected were modified to give even sampling across the field when sampling from irregular shaped fields and small sugarcane plots. In surveys of border rows, wetlands, waterways nearby sugarcane fields and large water bodies, 100 indigenous grass host plant stems were collected and inspected for borers and their natural enemies, as described above for sugarcane. In *Cyperus* spp. the insect is known to bore into the umbels and rhizomes (Conlong 1990). Hence, the umbels and rhizomes not covered with water were inspected. In large indigenous grasses, attention was given to the stalks only.

2.2.3 Data analysis, specimen handling and identifications

To determine the existence of any borer and/or natural enemy life stage, ten infested sugarcane plants (or 100 wild host plant stalks) per field were harvested, dissected and carefully examined for any borer and/or natural enemy life stage present. Any live stem borer stage found was collected and placed into a 30 ml plastic vial containing a piece of sugarcane stalk, sedge or artificial diet (Graham and Conlong 1988). The vial was sealed with a perforated lid. The perforation was covered with very fine mesh stainless steel gauze. Dead or diseased larvae, cocoons of parasitoids, predators and pupae were placed in empty 30ml plastic vials, and sealed with the perforated lid. The vials were numbered. These numbers corresponded with numbers on a data sheet, where relevant information about the samples collected was recorded. These data included information on host type,

part of the plant where the specimen was found, amount of damage, name of the organism if known, developmental stage, and date and area of collection.

The number of internodes in the sampled plants were counted and divided into three catagories: the third of the internodes towards the tip of the stalk was referred to as Upper one third; the part in the lower side with one third of the total internodes was referred to as Bottom one third; and the part between these two parts was referred to as Middle one third. The percentage of boring in a particular part of the stalk was obtained by dividing the total number of bored internodes in that part by the total number of bored internodes in the whole stalk and this multiplied by a hundred. Similarly, the proportion of internodes bored was calculated by dividing the total number of bored internodes counted, into the total internodes of the sample plant, and this value was multiplied by a hundred to get the percentage damaged internodes per plant. Pest incidence was obtained by dividing the proportion of infested sugarcane plots by the total number of plots sampled, and this value was translated into a percentage. Relative abundance of each stem borer species was determined as the total number of that species, expressed as the percentage of the total population (number) of all stem borer species found at each estate.

The collected specimens were shipped to the South African Agricultural Research Council's Plant Protection Research Institute (ARC-PPRI) Quarantine Laboratory in Pretoria, where they were reared through to either borer or parasitoid adults. Adults were identified by staff of the Biosystematics Division of the ARC-PPRI in Pretoria, and Dr D. Barraclough, School of Biological and Conservation Sciences, University of KwaZulu-Natal, Durban, South Africa. Voucher specimens of species identified are kept at the South African Sugarcane Research Institute (SASRI), Mount Edgecombe, KwaZulu-Natal, South

Africa and sequences of sample specimens used for barcoding (Chapter 3.3) were submitted to GenBank.

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Way M.J., Kfir R. 1997. The stem borer *Chilo partellus* (Swinhoe) (Lepidoptera, Pyralidae) in sugarcane in southern Africa. *African Entomology.* 5(1): 170-172.

2.3 STEM BORER COMPLEX (LEPIDOPTERA: NOCTUIDAE; CRAMBIDAE; PYRALIDAE) IN SUGARCANE ESTATES OF ETHIOPIA, THEIR HOST PLANTS AND NATURAL ENEMIES

2.3.1 Abstract

Surveys were completed in the three sugarcane estates of Ethiopia in 2003 and 2004 to assess the lepidopteran stem borer species in sugarcane and in wild host plants on the estates. A total of 174 sugarcane plots, and several patches of indigenous wild host plants in irrigation canals and field borders were evaluated for possible infestation by stem borers and for the existence of their natural enemies. The surveys verified the presence of three lepidopterous stem borer species on estate sugarcane. These were *Busseola* sp., *Chilo partellus* and *Sesamia calamistis*. *Eldana saccharina* was recovered from indigenous sedges in irrigation canals of the sugarcane estates of Metehara and Wonji. The *Busseola* sp. was the predominant stem borer of sugarcane on these two estates. *Chilo partellus* and *S. calamistis* were recovered in very low numbers at all three estates. Three parasitoid species, *Cotesia flavipes*, *C. sesamiae* (both from larvae collected at Wonji estate), and *Linnaemya* sp. (from Finchawa estate) emerged from field-collected stem borer life stages. Two fungal species and a bacterial pathogen were also isolated from the larvae of the borers found.

2.3.2 Introduction

Sugarcane for chewing has been grown in Ethiopia for many years (EARO 2000), however, production of refined sugar is of recent origin. Commercial cultivation of sugarcane in the country was started in the early 1950's at Wonji sugar estate (located in the Rift Valley about 100 km Southeast of the capital Addis Ababa) (Figure 2.3) (EARO 2000). Commercial plantations were extended to Metehara in the 1960's (EARO 2000).

These two commercial state farms are about 100km away from each other and are located in the Rift Valley at the upper course of the Awash river which is their sole source of irrigation water. The third estate, Finchawa, is the youngest commercial estate, and is located about 330km northwest of the capital, Addis Ababa. This estate was established in the early 1990's. Finchawa estate is the only commercial sugarcane estate found out of the Rift Valley and it is irrigated from the Finchawa river. At present, commercial production of the crop in the country is restricted to the three Government-owned estates that grow sugarcane in a total of 24000 ha.
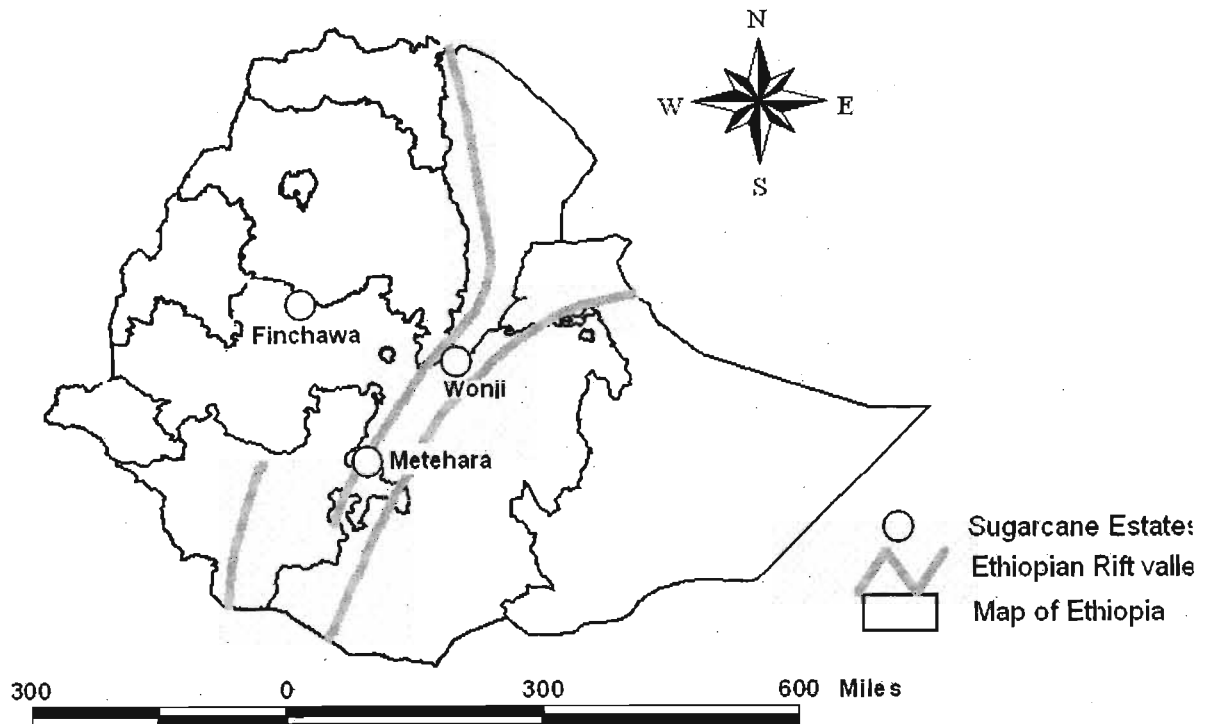
Figure 2.3   Map of Ethiopia showing locations of commercial sugarcane estate farms surveyed

Damage by lepidopteran stem borers in sugarcane has long been recognized in Ethiopia, but research data on species composition and natural enemy complex were lacking, being limited to internal reports of each estate, and confidential consultant reports. Thus, the current surveys were conducted to scientifically identify the stem borer species and their natural enemy complexes, in the commercial sugarcane estates of Ethiopia.

2.3.3   Materials and Methods

2.3.3.1 Survey sites

See Chapter 2.2.1.1

2.3.3.2 Survey methods

See Chapter 2.2.2.1 and 2.2.3

2.3.4 Results

2.3.4.1 Stem borer identification, distribution and abundance

Four lepidopterous stem borers were recorded from the three sugarcane estate farms of Ethiopia. They were identified as a *Busseola* sp. (Lepidoptera: Noctuidae), *Chilo partellus* Swinhoe (Lepidoptera: Crambidae), *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) and *Eldana saccharina* Walker (Lepidoptera: Pyralidae). Based on the data obtained from the two surveys in 2003 and 2004, the incidence of stem borers in sugarcane is relatively low (from 41.3% to 63.5%) as compared to the level of borers usually reported from sorghum and maize fields (Table 2.3).

Species composition and abundance varied from estate to estate (Table 2.3). In all the estates, sugarcane was infested by two to three species of stem borers. At Wonji, a complex of three stem borer species (*S. calamistis, C. partellus* and *Busseola* sp.) was found. In Metehara only two of these, *S. calamistis* and *C. partellus* were recorded from sugarcane. *Busseola* sp. was found to be the predominant stem borer at Finchawa and Wonji estates, followed by *S. calamistis* (Table. 2.3). *Chilo partellus* was found at all the estates but its abundance at Metehara and Finchawa estates was low (Table 2.3). *Eldana saccharina* was recovered only from the indigenous sedge, *Cyperus dives* C.B.Cl. (Cyperaceae) in irrigation canals at Wonji and Metehara estates. Sugarcane and other indigenous wild hosts evaluated were free from infestation by this borer (Table 2.3).

Table 2.3   Species composition and mean percentage incidence (±SE) of the stem borer species collected in 2003 and 2004 on sugarcane and indigenous wild host plants at the three commercial sugarcane estates.

Estate	No. of Fields	Host plant	Incidence (%)	No. of plants dissected	No. of borers recovered	Species composition (%) C. partellus	Busseola sp.	S. calamistis	E. saccharina
Wonji	74	Sugarcane	63.5±5.6	740	43	25±6.7	57±7.7	18±6.0	0
	4	C. dives	50±28.9	200	11	0	0	0	100
	5	T. latifolius	0	0	0	0	0	0	0
Metehara	46	Sugarcane	41.3±7.3	460	3	33±33.3	0	67±33.3	0
	7	C. dives	43±20.2	700	3	0	0	0	100
Finchawa	56	Sugarcane	53.6±6.7	560	16	6±6.3	69±11.9	25±11.2	0
	6	S. arundinaceum	0	0	0	0	0	0	0

Very high stem borer infestations (up to 35%) were recorded in young sugarcane (1 to 3 months old) plots at Wonji estate. However, infestation in older sugarcane was relatively lower at all estates. The mean percentage infestation ranged from 7% in Metehara to 17% in Wonji (Table 2.4). The percentage damaged internodes of infested stalks ranged from 7.4 to 10.1%. Although borings were found along the whole length of mature sugarcane stalks sampled, most were concentrated in the bottom and middle thirds of the stalks (Table 2.4).

Table 2.4 A summary of infestation (±SE) by stem borers recorded in the surveys conducted during 2003-2004 on the three Ethiopian sugarcane estates (U=Upper one third; M=middle one third; B=Lower one third.).

Estate	Alt (m.a.s.l.)	Age (Months)	% inf. Stalks	Mean No. nodes / Stalk	% nodes dam.	Part of the stalk bored (%)		
						U	M	B
Wonji	1500	±12	17±1.4	16±0.23	10.1±0.8	9±2.5	33±4.2	58±4.3
Metehara	960	±12	7±1.2	17±0.28	7.9±1.7	6.5±5.8	25.8±10.6	67.7±11.4
Finchawa	1635	±11	12±1.3	17±0.25	7.4±1.7	15±8.5	36.7±11.3	48.3±11.8

2.3.4.2 Natural enemies

All natural enemies obtained are shown in Table 2.5. Two larval parasitoids, the native *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) and the exotic *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), and a pupal parasitoid, *Linnaemya sp.* (Diptera: Tachinidae), were reared from stem borers collected. The level of parasitism by these indigenous and exotic parasitoids at the time of the surveys was very low, ranging from 2.3 to 6.3% (Table. 2.5).

The bacterial pathogen, *Bacillus thuringiensis* Berliner (Eubacteriales: Bacilliaceae), was found to be an important mortality factor of larvae of all stem borer species in young sugarcane at Wonji estate. Up to 50% of the larvae in young sugarcane (1 to 3 months old) were found killed by this bacterium with an overall mean percentage mortality of 34.9% of the larvae in this estate. Fungal pathogens, *Entomophthora* sp. (Entomophthorales:

Entomophthoraceae) and *Beauveria bassiana* Balls. (Deuteromycotina: Hyphomycetes), were also recorded but their impact on borer populations was minimal (Table 2.5).

Table 2.5 Natural enemies of stem borers recorded in the surveys conducted during 2003 and 2004 in sugarcane and indigenous sedges on the Ethiopian sugarcane estates.

Estate	Natural enemy found		Life stage attacked	Host plant	% Par.
	Species	No.			
Wonji	*C. flavipes*	1	Larvae	Sugarcane	2.3
	C. sesamiae	2	Larvae	Sugarcane	4.7
	B. thuringiensis	15	Larvae	Sugarcane	34.9
	Entomophthora sp	1	Larvae	Sugarcane	2.3
Finchawa	*Linnaemya* sp.	1	Pupae	Sugarcane	6.3
	B. bassiana	1	Larvae	Sugarcane	6.3

2.3.5 Discussion

2.3.5.1 Stem borer distribution and abundance

Insects associated with sugarcane are believed to be local insects that have adopted sugarcane as a host consequent to its cultivation (Pemberton and Williams 1969; Conlong 1994). The stem borer complex recorded in Ethiopian sugarcane estates is quite different from what was reported from other African countries (Leslie 1994; Conlong 1994). Of the four stem borers recorded in these surveys, two of the species, *Busseola* sp. and *C. partellus,* have never been considered important pests of sugarcane (Polaszek and Khan 1998; Charpentier and Mathes 1969; Way and Kfir 1997). In addition, the most important

African sugarcane borers, *S. calamistis* and *E. saccharina* (Leslie 1994) are of minor or no importance in Ethiopian estates. *Eldana saccharina* is confined only to indigenous host plants and *S. calamistis* is of minor importance at all the three estates with only few larvae collected from all the estates.

Busseola sp., *S. calamistis* and *C. partellus* were previously reported to be the predominant stem borer of sorghum and maize in highland, mid altitude and lowland areas of Ethiopia, respectively (Gebre-Amlak 1985; Getu *et al.* 2001). However, more recent surveys in Ethiopian peasant sugarcane farms have shown that these borers are also predominant in sugarcane (Chapter 2.4 and 2.5). The expansion in the range of host plants of these stem borers may be associated with mixed cropping practices followed in the country. As all the commercial estate farms visited are located in the middle of the major maize and sorghum growing regions of the country, sorghum and maize fields nearby might have played a role, as they did in the peasant situation, as an infestation source of stem borers now attacking sugarcane on the estates.

Cyperus dives, a well-known host plant of *E. saccharina* in other parts of Africa (Girling 1972; Atkinson 1979; Atkinson 1980; Conlong 2001), was common on Ethiopian sugarcane estates. As expected, populations of this borer were found in this sedge. In contrast, other recorded hosts such as *Typha latifolius* Moench (Typhaceae) and *Sorghum arundinaceum* (Desv.) Stapf. (Poaceae) that were reported to host *E. saccharina* in southeastern and West Africa (Girling 1972; Betbeder-Matibet 1981; Maes 1998; Polaszek and Khan 1998; Mazodze and Conlong 2003) were free from the pest in Ethiopia.

The presence of *E. saccharina* in indigenous host plants growing in irrigation canals of the estates is a potential danger for commercial sugarcane production. In Zimbabwe, where the

borer was first observed in sedges close to sugarcane in 1987, a severe outbreak of *E. saccharina* in sugarcane by *E. saccharina* was reported from two fields in 1999 (during a severe drought), and has since spread throughout their industry (Mazodze *et al.*, 1999; Mazodze and Conlong 2003). The same may happen in Ethiopia should current biotic and/or abiotic factors change to favor the incursion of *E. saccharina* into sugarcane. Climate, sugarcane expansion and related agronomic factors should continually be monitored in order to predict changes that favour infestation of sugarcane by *E. saccharina*, and to take corrective action before serious infestation occurs. The sugar estates in Ethiopia should implement proper montoring and be aware of the impact of crop management measures such as use of low nitrogen levels and early harvesting, to minimize the chance of *E. saccharina* colonization and population build-up in sugarcane. *Eldana saccharina* prefers older sugarcane when nutrients are no longer used for plant growth, especially nitrogen (Nuss *et al.* 1986), which then becomes available for insect use. It has also been shown that *E. saccharina* infestations increase as nitrogen fertilizer application rates increases (Carnegie 1981). Reduction of nitrogen fertilizer to 30kg per hectare is recommended to reduce *E. saccharina* problems in sugarcane (SASA 1994).

2.3.5.2 Natural enemies

Previous studies in sorghum and maize fields of Ethiopia (Gebre-Amlak 1985; Yitaferu and Walker 1997; Getu *et al* 2001) reported a large number of parasitoids associated with stem borers. However, only few of these were recorded in this study, perhaps because of the narrow window of sampling. The braconid *C. flavipes* was the only exotic parasitoid of stem borers recorded in this study. It is a gregarious endoparasitoid of lepidopterous stem borers of gramineous plants indigenous to South-East Asia (Mohyuddin 1971). This larval

parasitoid was introduced from Pakistan into Kenya (Overholt *et al.* 1994) from where it was released into other African countries (Overholt 1998). The parasitoid has never been released in Ethiopia, but it was recently found established on *C. partellus, Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) and *B. fusca* in maize and sorghum (Getu *et al.* 2001) and more recently a high level of parasitism of *C. partellus* by this parasitoid was recorded in peasant sugarcane farms at Girana (39°43′E; 11°34′N) in northern Ethiopia (Chapters 2.4 and 3.4).

The other parasitoids recorded in these surveys are the indigenous braconid larval parasitoid, *C. sesamiae,* and the tachinid pupal parasitoid *Linnaemya* sp. The former was reported to be a common parasitoid of stem borers in cereal grains in Ethiopia (Getu *et al.* 2001) whereas the later is reported only from sugarcane in the country (Assefa *et al.* 2006). This may indicate the existence of diverse parasitoid species in sugarcane fields of the country. Knowledge on the diversity and identification of the key biocontrol agents is important for proper management of stem borers in sugarcane fields of Ethiopia, and regular surveys throughout the year are needed to investigate their seasonal impact on borer populations. This also applies to the entomopathogens discussed below.

Baccillus thuringiensis was found to be an important stem borer mortality factor in young sugarcane fields at Wonji. Some isolates of *B. thuringiensis* are reported to be highly toxic to stem borer larvae (Jacobs 1989). Therefore, it is important to test the effect of the *B. thuringiensis* isolates found in Ethiopian sugarcane on the major stem borers for possible use of this pathogen in their management.

The fungal conidiophores that emerged from larvae killed by the entomopathogenic Entomophthorales fungus indicated that these pathogens are part of the natural enemy complex of stem borers in Ethiopian sugarcane. Several species of Entomophthorales are known to attack insects belonging to different orders (Hatting 2002). In recent surveys in Ethiopia, these entomopathogenic fungi were recorded from *E. saccharina* in sedges and from *Busseola* spp. in sugarcane (Assefa *et al.* 2006). *Beauveria bassiana,* which was reported from *E. saccharina* in South and West Africa (Conlong 1990, 2001) was also recorded in this study. The recovery of Entomophthorales fungi and *B. bassiana* in Ethiopian sugarcane may indicate the potential of these fungal pathogens to adapt to different habitats and attack diverse species of stem borers. The Entomophthorales fungal isolates need to be identified to species level and investigated as potential biocontrol agents.

The low number of parasitoid species recorded is ascribed to the low number of stem borer life stages recovered at the time of year the surveys were undertaken. There is a need to conduct a year round survey in all the estate farms in order to understand the population dynamics of the stem borers and their natural enemies. Such studies will be useful to understand the key natural enemies that are keeping the pest in check (Conlong 1990). Natural enemies with high potential as biocontrol agents could be used in the management of the economically important borers in sugarcane fields of the country.

2.3.6 Conclusions

These surveys show that a complex of stem borers has colonized sugarcane in commercial estates of Ethiopia, and there is a threat that *E. saccharina* could also invade Ethiopian

sugarcane. The natural enemy complex and the level of parasitism recorded are very low. The strain of *B. thuringiensis* found at Wonji estate needs to be identified and tested against all stem borers for its future potential in stem borer management in sugarcane in the country.

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2.4 *BUSSEOLA* SPECIES COMPLEX (LEPIDOPTERA: NOCTUIDAE) IN PEASANT SUGARCANE FARMS OF ETHIOPIA

2.4.1 Abstract

Surveys conducted in 2003 and 2004 in 45 peasant farmer fields in three administrative region of Ethiopia indicated that *Busseola fusca* Fuller (Lepidoptera: Noctuidae) and *Busseola phaia* Bowden (Lepidoptera: Noctuidae) are the major and most widely distributed sugarcane stem borers in these farms. Over 70% of the peasant sugarcane fields visited were infested by these borers, with the highest levels of infestation (35% and 50%) being in the northern and western part of the country, respectively. The natural enemy complex observed in sugarcane fields was completely different from that previously reported in cereal grain fields of the country. An unknown solitary hymenopteran larval parasitoid, a solitary dipteran pupal parasitoid *Linnaemya* sp., a fungus as well as the bacteria *Bacillus thuringiensis* were recovered from the two *Busseola* species in sugarcane and in Napier grass (*Pennisetum purpureum*). Agronomic practices that may contribute to the colonization of sugarcane by the borers are discussed.

2.4.2 Introduction

Sugarcane, *Saccharum* spp. L. (Poaceae) is a perennial crop widely grown in the tropical and subtropical areas of the world, including several countries in Africa, the Mascarene Islands and Madagascar (Overholt *et al.* 2003). Sugarcane is the sole source of sugar in Ethiopia and more than 36000 ha of land is allocated for production of this crop, of which about 35% is owned by peasant farmers. Sugarcane production in peasant farms of

Ethiopia typically occurs on smallholdings with a mean land size of approximately 0.02 ha. Peasants grow sugarcane in their gardens and/or in the field under irrigation. In these farms, sugarcane is planted near or mixed with other crops such as sorghum, maize, coffee, fruit and vegetables. The produce of these farmers is mainly used for food and cash crops. Sugarcane stalks are sold for chewing. None of the produce is supplied to sugar mills. An estimated 5144, 1013 and 6679 hectares of land is allocated for small-scale sugarcane production in the regions of Oromia, Amhara and Southern Nations Nationalities and Peoples' Region (SNNPR), respectively (EASE 2003).

As is the case in small farms in East Africa, sorghum and maize are staple foods for millions of Ethiopians and large areas of land are allocated for the cultivation of these cereals (FAO 2004). More than half of the maize and sorghum farmers in Ethiopia practice mixed cropping (Getu *et al.* 2001) and it is not uncommon to see sorghum and/or maize intercropped with/or planted in the vicinity of sugarcane. These practices have been reported to make the agro-ecosystem favourable for colonization by a number of pests (Lawani 1982). In addition, dry stalks of maize and sorghum are often used for building houses and fences, as fuel and bedding for livestock and are stacked in fields, where they are left for a long period until used. It was recognized earlier that these crop residues constitute an important reservoir of stem borers that give rise to new infestations (Ingram 1958; Gebre-Amlak 1988a; Päts 1996).

Lepidopteran stem borers are the most important pests of sorghum and maize in Ethiopia (Gebre-Amlak 1985, 1988b). Three indigenous and one exotic species of stem borer are known to attack sorghum and maize (Gebre-Amlak 1985) of which the noctuid *Busseola fusca* Fuller is reported to be the most serious pest at higher elevations (1160-2500m) and

the cooler areas of the country (Gebre-Amlak 1985). *Busseola fusca* is indigenous to Africa and is a serious pest of cereal grains (Harris 1962; van Rensburg *et al.* 1987). It is also a major pest of maize and sorghum in all African countries south of the Sahara (Harris 1989; Abate *et al.* 2000). It feeds on several wild grasses and crops (Ingram 1958; van Rensburg and van den Berg 1990; Haile and Hofsvang 2002; Midega and Khan 2003). It has been recorded from sugarcane fields in different parts of Africa, but never at pest levels (Polaszek and Khan 1998; Conlong 2000). In contrast to *B. fusca, Busseola phaia* Bowden has never been reported as a pest in crop fields. In the results of an extensive survey reported by Nye (1960), *B. phaia* was common in wild host plants and rarely found in maize fields adjacent to infested wild host plants. Recent surveys in Kenya, however, showed that *B. phaia* is becoming common in maize fields suggesting the potential of this insect to turn into a serious pest (Le Rü personal communication). This insect has never been recorded from sugarcane and its presence in Ethiopia has not been reported previously. The levels of infestation by the two *Busseola* species in Ethiopian peasant sugarcane were unexpectedly high and widespread. This chapter examines the status of the *Busseola* species complex in peasant sugarcane fields in Ethiopia and briefly discusses some agronomic practices that may contribute to the higher levels and widespread nature of infestations observed.
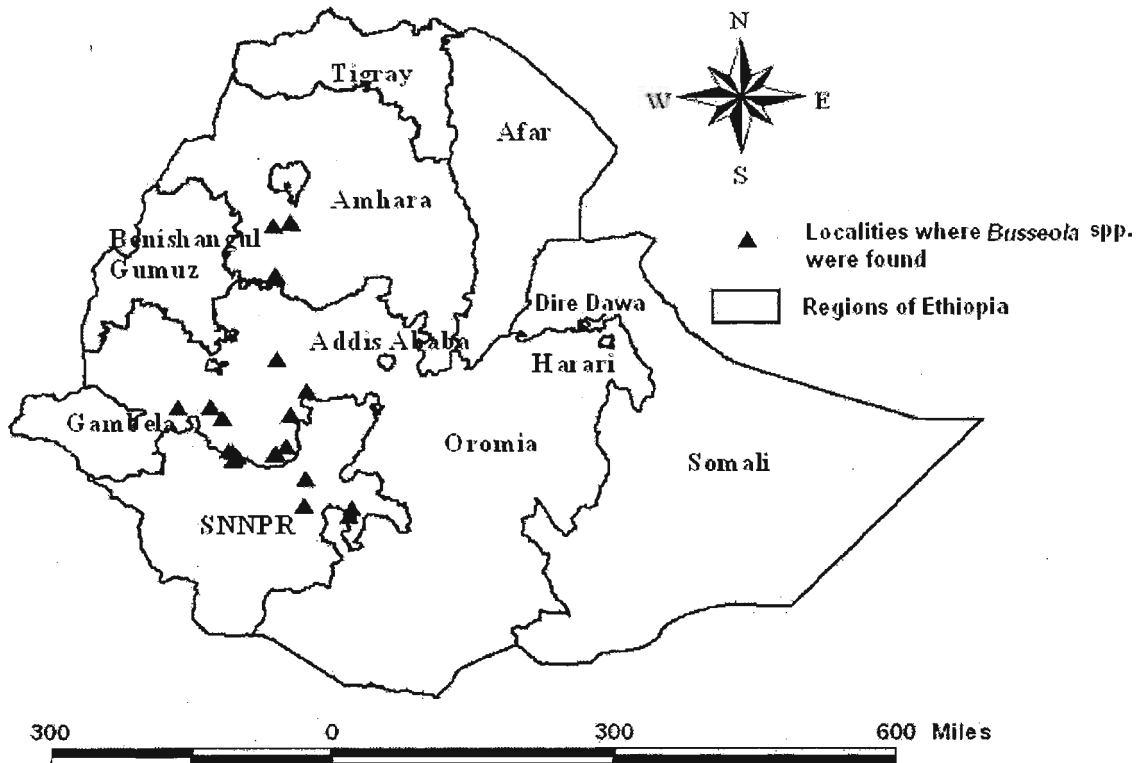
Figure 2.4   Map of Ethiopia showing localities where sugarcane was infested with *Busseola* species. Regions of Ethiopia are named. Locality names are indicated in Table 2.6.

2.4.3   Materials and Methods

2.4.3.1 Survey areas

See section 2.2.1.2

2.4.3.2 Survey methods

See sections 2.2.2.2 and 2.2.2.3

2.4.4 Results

2.4.4.1 Busseola *species in sugarcane fields of peasant farmers*

Thirty-two of the forty-five peasant farmers' fields visited were infested by *Busseola* species (Table 2.6 and 2.7). These were widely distributed at higher altitudes, from 1520m to 2060m, in all three regions included in surveys. Two *Busseola* species were found to be the predominant stem borers in all SNNPR fields, and comprised 75% of the borer complex in Oromia Region fields and 53% in Amhara Region's fields. The borers were particularly abundant in localities near Finote Selam in Amhara Region and in peasant farms near Jima in the Oromia Region (Figure 2.4, Table 2.7).

Table 2.6 Summary of surveys on peasant sugarcane farms during 2003 and 2004 for *Busseola* species and their natural enemies in the major sugarcane producing regions of Ethiopia

Region	Altitudinal Range (Meters above sea level)	No. of peasant farms visited	Status of *Busseola* Present	Absent
Oromia	1410-2060	20	15	5
Amhara	1310-1930	17	9	8
SNNPR	1640-1880	8	8	0

Very high infestations of these species were recorded at two sites in Amhara and Oromia Regions, one at Mankusa in December 2003 and the other at Bedaye in December 2004 respectively (Table 2.7). The percentage damaged internodes per infested stalk ranged from 5.5 to 25.3%. Although borings were found along the whole length of sugarcane stalks sampled, most were concentrated in the upper and middle thirds of the stalks (Table 2.7). Sixty-three percent of the borings observed were on the upper one-third of the stalks; 32% were in the middle one-third and only 5% of the borings were found in the bottom third of the stalks. Larvae and pupae of *Busseola* species were recovered only from the upper and middle thirds of sugarcane stalks and no borers of any species were recovered from the bottom of the stalks.

Table 2.7 A summary of infestation (±SE) by *Busseola* species recorded during 2003 and 2004 in peasant sugarcane farms of Ethiopia (U=upper one third; M=middle one third; B=lower one third.). Names in bold are names of the regions.

Region	Locality	Position	Alt (m)	Age (months)	% infested stalks	Mean No. nodes / Stalk	% nodes damaged	Part of the stalk bored (%) U	M	B
Amhara	Weyiniye	37°33′E; 11°29′N	1840	±16	5±2.1	21±2.8	6.8±1.7	57.1±13.7	42.9±13.7	0
	Mankusa	37°11′E; 10°40′N	1880	±11	20±4.0	12±2.2	9.8±2.8	100	0	0
	Mankusa	37°10′E; 10°42′N	1930	±12	15±3.6	15±0.5	14.6±2.9	6.7±4.5	53.3±10.1	40±10.7
	Mankusa	37°58′E; 08°50′N	1890	±23	25±5.1	26±2.3	11.7±1.9	73.1±8.2	26.9±8.2	0
	Mankusa	37°11′E; 10°40′N	1850	±17	30±4.6	25±2.2	16.0±2.3	100	0	0
	Mankusa	37°11′E; 10°40′N	1875	±15	35±4.8	32±3.4	18.4±2.2	90.6±3.9	9.4±3.9	0
	Ingutti	37°06′E; 11°24′N	1895	±18	14±4.9	14±2.9	6.3±2.1	57.1±17.6	42.9±17.6	0
	Tis Abay	37°35′E; 11°29′N	1600	±12	15±3.6	11±0.7	9.2±2.8	36.4±16.3	63.6±16.3	0
	Mendal	37°35′E; 11°29′N	1605	±6	10±4.3	12±1.1	8.2±2.5	25±13.3	66.8±15.3	8.3±10
Oromia	Oda haro	37°12′E; 09°03′N	1600	±10	25±0.4	23±1.2	20.9±3.3	82.6±5.4	17.4±5.4	0

Region	Locality	Position	Alt (m)	Age (months)	% infested stalks	Mean No. nodes / Stalk	% nodes damaged	Part of the stalk bored (%)		
								U	M	B
Oromia										
	Lale Belo	36°43′E; 07°48′N	1715	±9	15±0.4	15±0.8	17.2±	86.7±6.1	13.3±6.1	0
	Bedaye	37°31′E; 08°09′N	1700	±6	25±4.4	16±1.1	18.6±3.1	50.0±9.3	50.0±9.3	0
	Bedaye	37°31′E; 08°09′N	1690	±9	50±5.0	21±1.4	25.3±3.0	90.5±4.1	9.5±4.1	0
	Bedaye	37°31′E; 08°09′N	1700	±10	20±8.2	13±0.9	13.4±2.9	100	0	0
	Sanbo	35°58′E; 08°23′N	1520	±22	15±3.7	16±1.2	12.5±3.1	75.0±9.9	25.0±9.9	0
	Shengela	36°08′E; 08°23′N	1820	±12	20±4.2	19±0.9	11.6±2.3	89.5±6.3	10.5±6.3	0
	Dheka Tubo	36°09′E; 08°22′N	1870	±12	32±4.8	17±0.6	13.3±2.6	94.1±4.3	5.9±4.3	0
	Geate	36°09′E; 08°22′N	2060	±22	21±4.1	22±0.9	17.1±2.6	100	0	0
	Dembi	36°28′E; 08°05′N	1875	±22	2±2	21±1.3	16.3±2.5	95.2±4.1	4.8±4.1	0
	Goma	36°36′E; 07°51′N	1580	±12	12±3.3	18±0.9	11.3±2.3	83.3±8.2	11.1±6.7	5.6±5
	Gebe Buso	37°08′E; 07°44′N	1620	±22	25±4.4	15±1.2	7.8±2.2	100	0	0
	Koch 03	36°51′E; 07°41′N	1640	±16	30±4.6	22±2.1	9.3±1.9	59.1±11.4	40.9±11.4	0

egion	Locality	Position	Alt (m)	Age (months)	% infested stalks	Mean No. nodes / Stalk	% nodes damaged	Part of the stalk bored (%)		
								U	M	B
)romia	Buyo Challa	36°45′E; 07°37′N	1670	±12	5±2.2	13±1.2	10.8±2.7	76.9±11.4	23.1±11.4	0
	Sika Cheqorsa	36°43′E; 07°35′N	1760	±13	5±2.2	10±1.1	5.5±2.4	0	100	0
	Kelta	37°26′E; 07°57′N	1670	±12	30±4.6	20±1.4	13.7±2.4	40.0±9.6	55.0±9.7	5.0±3.7
NNPR	Kelta	37°26′E; 07°57′N	1660	±11	20±4.0	18±1.0	16.2±2.7	27.8±8.4	61.1±9.2	11.1±5.7
	Welayita Sodo	37°53′E; 06°59′N	1880	±12	25±4.4	14±0.8	9.6±2.5	35.71±14	64.3±14	0
	Gachano	37°55′E; 07°02′N	1830	±11	5±2.2	15±1.2	7.0±2.1	0	33.3±15.2	66.7±15.2
	Gachano	37°55′E; 07°02′N	1810	11	5±2.2	18±1.1	12.8±2.4	0	94.4±4.8	5.6±4.8
	Sidama	38°26′E; 06°54′N	1870	±12	35±4.8	17±1.4	9.8±2.3	82.4±9.5	17.6±9.5	0
	Sidama	38°21′E; 06°41′N	1770	±12	30±6.5	15±1.3	9.7±2.5	80.0±10.7	20.0±10.7	0
	Bela wajo	37°10′E; 07°45′N	1640	±12	5±3.5	14±1.2	14.3±2.9	50.0±11.5	50.0±11.5	0

2.4.4.2 Busseola *species in indigenous host plants*

In surveys of indigenous host plants growing adjacent to sugarcane fields, Napier grass,

Pennisetum purpureum Moench (Poaceae), planted on borders of a peasant sugarcane farm

at Mankusa was found infested by *Busseola* spp. Old and new borings were found in 15% of the sample stalks inspected. Three borer larvae were recovered, of which one was parasitized by a hymenopteran solitary parasitoid (Table 2.8).

2.4.4.3 *Natural enemies of* Busseola *species*

In visits made to peasant sugarcane farms in the three major sugarcane-producing regions of Ethiopia, two parasitoid species and two pathogen species were reared from *Busseola* species collected in sugarcane and *P. purpureum* (Table 2.8). A tachinid parasitoid (*Linnaemya* sp.) emerged out of a noctuid pupa collected from sugarcane in Bedaye area in December 2004. In addition, a solitary hymenopteran larval parasitoid was recovered, being reared from a fifth instar larvae collected in Mankusa. In the current surveys *Entomophthora* sp., and *Bacillus thuringiensis* were found infecting larger larvae in sugarcane.

Table 2.8   Natural enemies found, and the level of parasitism recorded on *Busseola* species in sugarcane fields and bordering *Pennisetum purpureum* in peasant farms in Ethiopia

Location	Host plant		Life stage	Natural enemy found		
	Type	Age (month)	attacked	Species	No.	% paras.
Mankusa	Sugarcane	±23	Larva	Hymenopteran solitary parasitoid	2	25
Bedaye	Sugarcane	±10	Pupa	*Linnaemya* sp.	1	50
Bedaye	Sugarcane	±9	Larva	*B. thuringiensis*	2	22.2
Bedaye	Sugarcane	±9	Larva	*Entomophthora* sp.	1	11.1
Oda Haro	Sugarcane	23	Larva	*Entomophthora* sp.	2	33.3
Mankusa	*P. purpureum*	Mature	Larva	Hymenopteran solitary parasitoid	1	33.3

2.4.5 Discussion

2.4.5.1 Busseola *species in sugarcane fields of peasant farmers*

Growing two or more crops in the same field at the same time is a long-standing practice in traditional African agriculture (Abate *et al.* 2000). This practice has been reported to reduce levels of pest and disease incidence, and favour abundance of natural enemies (Amoaka-Atta 1983; Amoaka-Atta *et al.* 1983; Okeyo-Owuor *et al.* 1991). However the danger of population build-up may occur when companion crops share the same pests (Abate *et al.* 2000). In peasant farms of Ethiopia, sugarcane is usually grown on small plots surrounded by land planted with sorghum and maize, which are major hosts of stem borers (Gebre-Amlak 1985; Getu *et al.* 2001; Tefera 2004).

In the current sugarcane surveys, *Busseola* species were the predominant stem borers in higher altitude areas of the country (1520-2060 m.a.s.l.). In these areas, *B. fusca* was reported to be a serious pest of sorghum and maize in the country (Gebre-Amlak 1985). In addition, *Busseola* species were widely distributed in all the major sugarcane producing regions surveyed (Table 2.7). *Busseola fusca* is known to occur sporadically in sugarcane fields (Polaszek and Khan 1998) but high levels of infestation by this pest on sugarcane have not been recorded elsewhere. In contrast, *B. phaia* is restricted to wild graminaceous plants and rarely seen in cultivated crops such as maize (Nye 1960). The observed level of infestation by the *Busseola* spp. (up to 50%) in peasant sugarcane farms therefore may have arisen from colonization of sugarcane by the pest from sorghum, maize and wild host plants growing adjacent to or mixed with the crop. In studies on the effects of surrounding

crops on the incidence of stem borers in maize, Van den Berg and Rebe (2001) and Ndemah *et al.* (2000) observed that good hosts of stem borer larvae served as a source of infestation for adjacent crops. Similar results were obtained by Girling (1978) for the sugarcane borer, *Eldana saccharina* Walker (Lepidoptera: Pyralidae): old stems of maize and sorghum left standing after harvest led to re-infestation of the next crop. The frequent droughts that result in crop failure in Ethiopia may even further enhance colonization of sugarcane by pests. In years where annual crops are devastated by drought, *Busseola* spp. moths will have no alternative but to lay eggs on less favoured sugarcane plants (Polaszek and Khan 1998). Moreover, larval migration from dying stalks of maize, sorghum and wild hosts to sugarcane may occur in years of rain scarcity.

Stubble and old stems of sorghum and maize left in the field were shown to contain large numbers of live larvae and constituted an important reservoir for new infestations of lepidopteran stem borers (Ingram 1958; Girling 1978; Gebre-Amlak 1988b; Päts 1996). These residues have long been known to play an important role in survival of *B. fusca* throughout the dry season (Harris 1962; Gebre-Amlak 1988a). Destroying crop residues by burning has been recommended (Gebre-Amlak 1988a). However, this is not a desirable practice for peasant farmers in Ethiopia since they use dry stalks for fuel and as building material (Gebre-Amlak 1985). Hence, it is important to investigate alternative sanitation measures should non-destruction of crop residues be identified as the cause of high levels of *Busseola* spp. infestation on sugarcane.

Detailed studies on the sources and mechanisms of infestation by *Busseola* spp. on sugarcane will help determine strategies to prevent pests from invading sugarcane, and

develop sound management practices for these pests applicable in places where mixed cropping is practiced.

2.4.5.2 Busseola *species in indigenous host plants*

Recent studies have shown that many indigenous grass species are highly attractive to ovipositing female moths of stem borers, although survival of immature stages on many of them is close to zero (Shanower *et al.* 1993; Khan *et al.* 1997; Schulthess *et al.* 1997). It was thus concluded that indigenous host species could act as trap plants. This phenomenon was exploited by several researchers in the 'Push–Pull' strategy (Pyke *et al.* 1987; Khan *et al.* 1997; Van den Berg and Rebe 2001; Midega and Khan 2003), which involves trapping the pest on host plant species that are attractive for adult moth oviposition, but unsuitable for larval development, and driving them away from the crop using repellent intercrops (Pyke *et al.* 1987; Miller and Cowles 1990; Verkerk *et al.* 1998). In a study conducted in southern Ethiopia, Gebre-Amlak (1988b) identified *P. purpureum* and *Sorghum arundinaceum* (Desv.) Stapf. (Poaceae) as major hosts of *B. fusca* in Ethiopia, and suggested that abundance of these wild host plants constituted a large reservoir of *B. fusca*. Similar conclusions were made by Ndemah *et al.* (2000) in Cameroon where they found high survival of first instar larvae and possible migration of young larvae from *P. purpureum* onto maize.

However, wild host plants may have a net beneficial effect by providing additional habitat for natural enemies (Khan *et al.* 1997; Schulthess *et al.* 1997). Ndemah *et al.* (2001), working in the forest zone of Cameroon, found a higher parasitoid species diversity on *P. purpureum* than on maize. It was suggested that such indigenous host plants play an

important role in maintaining stable parasitoid populations during the off-season and thereby lower pest incidence in crop fields during the growing season (Ndemah *et al.* 2003). However, the use of *P. purpureum* as a trap crop in a push-pull strategy in the management of *Busseola* spp. in peasant sugarcane farms of Ethiopia depends on a thorough understanding of the role of this indigenous host plant in increasing the mortality of these pests, and maximizing the availability of natural enemies.

2.4.5.3 *Natural enemies of* Busseola *species in sugarcane fields*

One of the surprising results of the current surveys is the difference between the natural enemy guild attacking *Busseola* spp. in sugarcane fields compared to the natural enemies attacking *B. fusca* in cereal grain fields in the country, although this could also be a result of the narrow window of sampling completed during this study. However, Gebre-Amlak (1985) reported six hymenopteran parasitoids (two pupal and four larval) and a predator attacking *B. fusca* in sorghum and maize in south, south-western and eastern Ethiopia. In a survey conducted by Yitaferu and Walker (1997) in the eastern part of Ethiopia, three parasitoids were found attacking *B. fusca* larvae in cereal grains. More recently, Getu *et al.* (2001) and Tefera (2004) reported a large number of natural enemies to be associated with *B. fusca* in sorghum and maize fields. However, none of the natural enemies reported in these studies were found in sugarcane fields in the current surveys. The natural enemy complex recorded in sugarcane fields of the peasant farmers is completely different to what was observed in cereal grain fields of the country. All the natural enemies recorded in these sugarcane surveys are thus new records for the pest in Ethiopia.

Conlong (2001) observed a difference in host plant preference and natural enemy distribution in *E. saccharina* and suggested the possible existence of different biotypes. Similar events could have arisen in *B. fusca* in Ethiopia. It may also be possible that the natural enemy complex recorded in sugarcane fields are all associated with *B. phaia* which may have colonized sugarcane as a result of a possible host plant shift. Phylogenetic studies of *B. fusca* populations and investigations on the natural enemy complex of *B. phaia* species in its natural habitat would be important to allow informed decisions to be made regarding natural enemy selection and pest management in sugarcane fields.

2.4.6 Conclusions

Busseola spp. were found to be the predominant stem borer species in peasant sugarcane farms in high altitude areas of the country. The natural enemy complex recorded from *Busseola* species in sugarcane might have been associated with these species from their natural habitats. This, however, will only be proven by an investigation of the natural enemies governing population build-up of these insects in their natural habitats in Ethiopia.

The presence of *Busseola* spp. in *P. purpureum*, and the common occurrence of larval parasitoids in both sugarcane and the indigenous host plant at Mankusa indicate that a habitat management approach to its control is a real possibility in this area.

2.4.7 References

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2.5 *CHILO PARTELLUS* SWINHOE (LEPIDOPTERA: CRAMBIDAE) AND ITS NATURAL ENEMIES IN PEASANT SUGARCANE FARMS OF ETHIOPIA

2.5.1 Abstract

Surveys of sugarcane stem borers and their natural enemies in Ethiopia were undertaken in 2003 and 2004. The surveys showed *Chilo partellus* Swinhoe to be the predominant sugarcane stem borer in lowland areas of the Northern, Southern and Eastern parts of the country. This species is an important pest of sorghum and maize in warmer lowland regions of Ethiopia, but was not recorded before from sugarcane. This Chapter is the first report of the presence of *C. partellus* in sugarcane fields of Ethiopia and describes the natural enemies found attacking it in sugarcane fields at the time of the surveys. The exotic parasitoid, *Cotesia flavipes* Cameron, was found to be an important natural enemy of *C. partellus* larvae in northern Ethiopia, with up to 50% parasitism. An unidentified Scelionid egg parasitoid, and an *Entomophthora* sp. fungus were also collected from the eggs and larvae respectively in the fields.

2.5.2 Introduction

The spotted stem borer, *Chilo partellus* Swinhoe (Lepidoptera: Crambidae), is indigenous to Asia and was accidentally introduced into East Africa before the 1930's (Harris 1990). Since then it has spread to many countries in eastern, central and southern Africa (Overholt *et al.* 1994; Maes 1998). This stem borer proved to be highly competitive and colonized many areas in eastern and southern Africa, displacing indigenous stem borers (Kfir *et al.*

2002). Currently, *C. partellus* is a serious pest of sorghum and maize in many parts of Africa (Päts *et al.* 1997; Overholt *et al.* 1994; Seshu Reddy 1983, van den Berg *et al.* 1990) and it is also known from rice and sugarcane (Kfir 1992, 1993; Way and Kfir 1997).

The importance of *C. partellus* in sorghum and maize in the warm lowland regions of Ethiopia has been reported by Getu *et al.* (2001), Gebre-Amlak (1985) and Tefera (2004), but no information exists on the presence of this pest, and the performance of its natural enemies in other graminaceous crops in the country. The present study examines the status of *C. partellus* in sugarcane fields of peasant farms of Ethiopia and the diversity and extent of parasitism by the natural enemies in these farms at the time of the surveys.

2.5.3   Materials and Methods

2.5.3.1 Survey areas

See Chapter 2.2.1.2

2.5.3.2 Survey methods

See Chapters 2.2.2.2 and 2.2.2.3
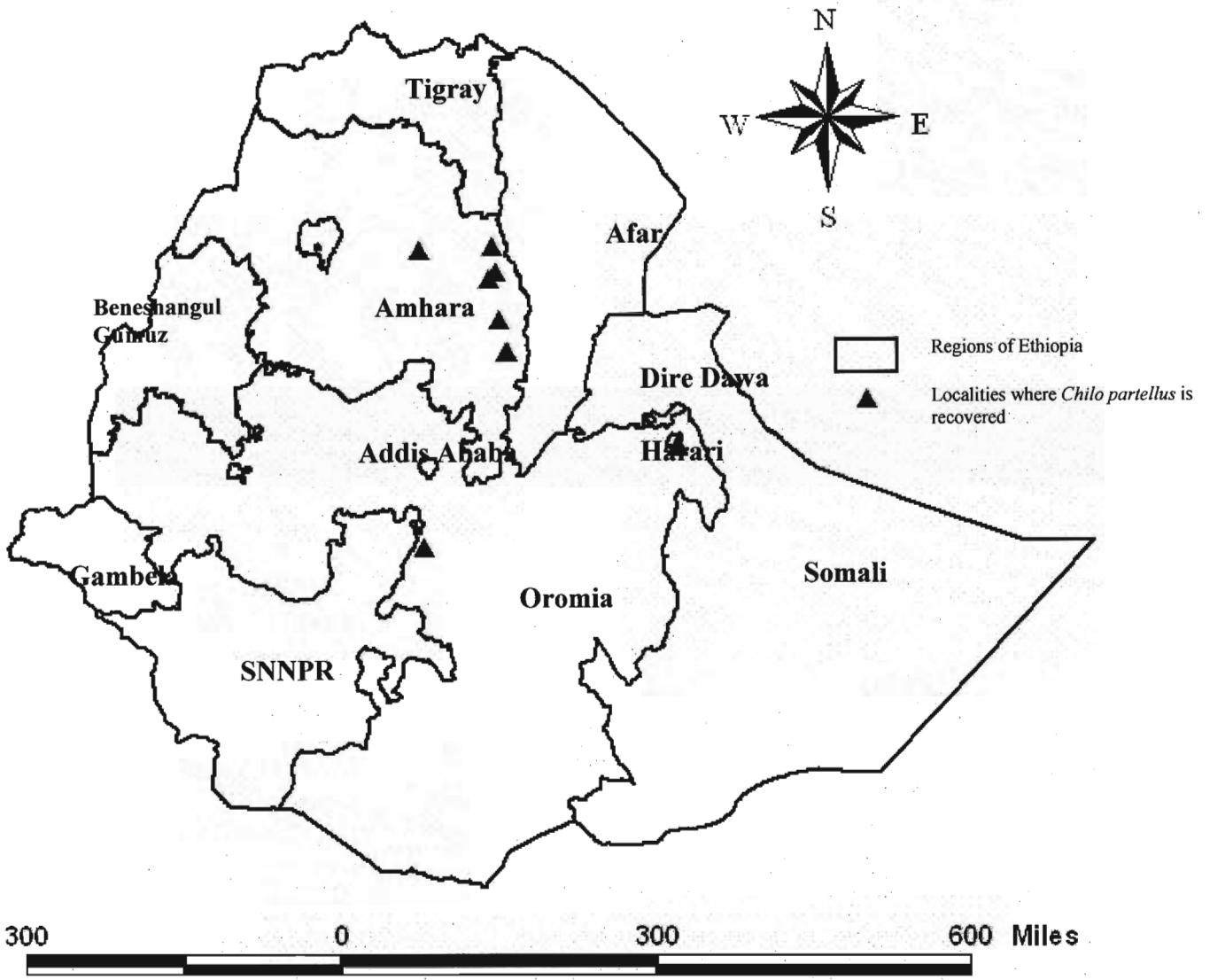
Figure 2.5 Map of Ethiopia showing localities where sugarcane was infested with *Chilo partellus*. Regions of Ethiopia are named. Locality names are indicated in Table 2.9.

2.5.4   Results

2.5.4.1 Chilo partellus *in peasant sugarcane farms*

Chilo partellus was found only in two of the three regions included in the surveys, Oromia and Amhara (Figure 2.5), occurring in the northern and eastern lowlands. None of the peasant farmers' fields surveyed in SNNPR were infested by this stem borer (Table 2.9). In December 2004, high infestations were recorded at two sites in Amhara region, one at Guba lafto (39°41′E; 11°54′N; 35%) and Girana (39°43′E; 11°34′N; 15%) (Table 2.9). The percentage of damaged internodes per infested stalks ranged from 4.69 to 17.86%. Although borings were found along the whole length of sugarcane stalks sampled, most were concentrated in the lower and middle thirds of the stalks. More than half of the borings (51.9%) observed were in the middle one-third of the stalks; 32.1% were in the lower one-third and only 16% of the borings were found in the upper third of the stalks. Larvae and pupae of *C. partellus* were recovered from all parts of the sugarcane stalks.

2.5.4.2 Natural enemies of C. partellus *in peasant sugarcane farms*

The natural enemies and the extent of parasitism recorded from *C. partellus* immatures is shown in Table 2.10. Two parasitoids and a pathogen were reared from *C. partellus* collected from sugarcane. High levels of parasitism (50%) of *C. partellus* larvae by *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) in sugarcane at Girana district in Northern Ethiopia is the first evidence of establishment of this natural enemy in sugarcane fields of the country. An unknown Scelionid egg parasitoid was also recovered for the first time in these surveys with a percent parasitism of 33.3 in sugarcane fields at Ziway (Table

2.10). Previous records on fungal and bacterial pathogens associated with stalk borers in the country are very scarce (Getu *et al*. 2001). In the current surveys, however, *Entomophthora* sp. (Entomophthorales: Entomophthoraceae) was found attacking *C. partellus* larvae in sugarcane at Girana.

Table 2.9 Summary of infestation (± SE) of *Chilo partellus* recorded in the surveys conducted during 2003 and 2004 in sugarcane fields of peasant farmers in various parts of Ethiopia. Names in bold are names of the regions. (U=upper one-third, M=middle one-third, B=lower one-third)

Locality	Position	Alt (m)	Age (Mths)	% inf. Stalks	Mean No. nodes/ Stalk	% nodes dam.	Part of the stalk bored (%) U	M	B
Amhara									
Guba lafto Site 1	39°41′E; 11°54′N	1350	±10	35±4.7	30.7±1.4	5.21±1.3	-	56.25±12.8	43.7512.8
Guba lafto Site 2	39°41′E; 11°51′N	1370	±17	10±4.3	30.7±1.7	8.51±1.6	5±3.8	80±7.9	15±7.2
Girana	39°43′E; 11°34′N	1400	±12	15±3.6	23.5±2.6	6.30±1.5	-	62.5±12.5	37.5±12.5
Tis Abalima	39°38′E; 11°28′N	1500	±17	5±2.2	38.1±1.6	4.69±1.1	100	-	-
Harbu	39°47′E; 10°55′N	1390	±8	10±4.3	32.0±1.4	5.88±1.3	-	20±9.6	80±9.6

Locality	Coordinates	Altitude	±						
Majete	39°53′E; 10°30′N	1525	±12	12±6.6	25.5±1.8	6.38±1.5	-	66.67±11.9	33.33±11.9
Shoa Robit	39°52′E; 10°30′N	1310	±17	10±4.3	28.2±2.1	6.67±1.5	-	50±11.8	50±11.8
Oromia									
Ziway	38°43′E; 07°55′N	1647	±12	5±2.2	11.2±0.9	17.86±3.6	30±10.5	50±11.5	20±9.2
Erer Site 1	42°14′E; 09°16′N	1329	±12	10±3.0	21.3±1.2	8.45±1.9	-	83.33±9.0	16.67±9.0
Erer Site 2	42°14′E; 09°15′N	1329	±12	10±3.9	24.7±1.4	8.10±1.7	25±9.9	50±11.5	25±9.9

Table 2.10   Natural enemies found and the level of parasitism recorded on *Chilo partellus* in the surveys conducted during 2003 and 2004 in sugarcane fields of peasant farms in Ethiopia.

Locality	Natural enemy recorded	Host stage attacked	No. of larvae/egg batches collected	No. of larvae/egg batches parasitised	% parasitism
Girana	*Entomophthora* sp.	Larva	16	1	6.25
Girana	*Cotesia flavipes*	Larva	16	8	50
Ziway	Scelionidae sp.	Egg	3	1	33.3

2.5.5 Discussion

2.5.5.1 Chilo partellus in peasant sugarcane farms

The exotic stem borer, *C. partellus*, has proven to be a highly competitive colonizer in many of the areas it has invaded, often becoming the predominant and most economically important stem borer species in maize and sorghum at elevations below 1800m (Seshu Reddy 1983). This insect has displaced the economically important indigenous stem borer species, *Busseola fusca* Fuller (Lepidoptera: Noctuidae) from maize fields in South Africa (Kfir 1997), and *Chilo orichalcociliellus* Strand (Lepidoptera: Crambidae) from maize in Kenya (Overholt 1998). The success of *C. partellus* in colonizing new areas and reaching economically important densities in Africa is not well understood (Overholt 1998), but its short generation time and high larval migration may play a significant role. In Ethiopia, *C. partellus* was previously reported to be the predominant stem borer of sorghum and maize in localities with altitudes of 1200 to 1690 m.a.s.l. (Gebre-Amlak 1985) more recent surveys, however, have shown that the insect is a predominant stem borer of maize and sorghum at an altitude of 1900 m.a.s.l. (Getu *et al.* 2001) to 1980 m.a.s.l. (Tefera 2004).

The current surveys revealed that *C. partellus* is expanding its host plant range to include sugarcane in areas within the limit of its altitudinal range (1310-1647 m.a.s.l.) in the country. *C. partellus* has never been reported as a predominant stem borer of sugarcane but is known to be an opportunist pest when sugarcane is planted near sorghum and maize fields (Charpentier and Mathes 1969; Way and Kfir 1997). In peasant farms of Ethiopia, sugarcane is produced in small plots in gardens and/or in the field under irrigation near or mixed with other graminaceous crops such as sorghum and maize. This practice has been

reported to make the agro-ecosystem favourable to a number of pests (Lawani 1982). In addition, dry stalks of maize and sorghum are often used for building houses and fences, as fuel and as bedding for livestock. They are stacked in fields to dry and left for long periods until used. These crop residues constitute an important reservoir of stem borers that gives rise to new infestations (Ingram 1958; Gebre-Amlak 1988; Päts 1996) in host plants growing in the area. As *C. partellus* is the dominant maize and sorghum stem borer in lowlands of the country (Gebre-Amlak 1985; Getu *et al.* 2001; Tefera 2004) these cereals are undoubtedly the sources of the *C. partellus* population found in sugarcane fields.

2.5.5.2 *Natural enemies of* C. partellus *in peasant sugarcane farms*

The braconid wasp, *Cotesia flavipes,* was the dominant parasitoid of *C. partellus* larvae in the Northern part of the country. *Cotesia flavipes* is a gregarious endoparasitoid of lepidopterous stem borers of gramineous plants indigenous to South-East Asia (Mohyuddin 1971). This larval parasitoid was introduced from Pakistan into Kenya in 1993 (Overholt *et al.* 1994) and it has become permanently established in the maize fields of the country (Omwega *et al* 1997; Songa *et al.* 2001). Following the success in Kenya, *C. flavipes* was released in Mozambique in 1996, and Uganda and Somalia in 1997 (Overholt 1998) and results showed that the parasitoid is attacking *C. partellus* in maize fields in these countries. Releases were later made in many countries in East and southern Africa and successful establishments by the parasitoid were reported from many of these, including Mozambique (Cugala and Owmega 2001), Tanzania (Omwega *et al.* 1997) and Uganda (Matama-Kauma *et al.* 2001). The parasitoid has never been released in Ethiopia, but it was recently found established on *C. partellus, Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) and *B. fusca* in maize and sorghum (Getu *et al.* 2001). This population is

speculated to be descendent of *C. flavipes* population released in Somalia and/or Kenya that expanded further north to Eastern and Northern Ethiopia (Getu *et al.* 2001, 2003). Molecular analysis of *C. flavipes* specimens from *C. partellus* in Ethiopian sugarcane revealed that this population is descendent of the same population released in Kenya and Somalia (see Chapter 3.).

These results may contradict the hypothesis of the parasitoid having "ecological races" (Mohyuddin 1991; Smith *et al* 1993). These authors reported the development of ecological races of *C. flavipes* that are adapted to searching different plants infested by stem borers. They provide the example of rice strain of *C. flavipes* from Pakistan that successfully attacked *C. partellus* in maize and sorghum, but did not attack *C. partellus* in sugarcane. The *C. partellus-C. flavipes*-sugarcane association in Ethiopia may indicate the potential of *C. flavipes* released against maize stem borers to act as a biocontrol agent for similar crambid sugarcane borers. This indicates that some populations of this parasitoid may have the ability to search different host plants for similar host borer species equally well, and thus may not be host and habitat specific. This effect can be negated though, as at least some pyralids and noctuids encapsulate *C. flavipes*. (Conlong 1997; Overholt *et al.* 1994). Potting *et al.* (1997) have also demonstrated that strains of *C. flavipes* differ in their ability to develop in species of stem boring lepidoptera primarly due to overcoming the encapsulation response of host species.

The other parasitoid found was a species belonging to the family Scelionidae (Hymenoptera), recovered from *C. partellus* eggs in Ziway. Several species of Scelionidae have been recorded as primary parasitoids of eggs of African cereal stem borers (Polaszek

and Kimani-Njogu 1998). However, there has been no report of the existence of these parasitoids from Ethiopian sugarcane. Their identification is still underway.

The fungal conidiophores that emerged from a *C. partellus* larva killed by the entomopathogenic Entomophthorales fungus indicated that this pathogen is part of the natural enemy complex attacking *C. partellus* in Ethiopian sugarcane. Several species of Entomophthorales fungi are known to attack insects belonging to different orders (Hatting 2002). In a study conducted on cereal aphids in South Africa, Hatting (2002) reported six species of Entomophthorales attacking aphids, of which three were characterized as major aphid-pathogenic species. Other Entomophthorales were also reported from *Eldana saccharina* Walker (Lepidoptera: Pyralidae) in South Africa (Carnegie 1987). In recent surveys in Ethiopia, these entomopathogenic fungi were recorded from *E. saccharina* in sedges and from *Busseola* spp. in sugarcane (Chapters 2.4 and 2.6). The presence of the Entomophthoran species on *C. partellus* indicates the potential of this Order of pathogens to adapt to different habitats and attack diverse species of stem borers. These pathogens need to be identified to species level and investigated as potential biocontrol agents.

2.5.6 Conclusions

Chilo partellus was found to be the predominant sugarcane stem borer in the warm lowlands of the Amhara and Oromia regions of Ethiopia. The insect has never before been reported to reach pest level status in sugarcane. The high level of infestation by this borer in Ethiopian sugarcane may be associated with the mixed farming system practiced by peasant farmers.

The presence of diverse natural enemies attacking *C. partellus* in sugarcane on these few sampling occasions is a good sign for the possibility of managing the pest using natural enemies. Investigations on the advantages and disadvantages of the current cropping systems that are being followed, and the dynamics of this pest and its natural enemies is essential to develop a sound *C. partellus* management strategy in sugarcane.

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2.6 STATUS OF *ELDANA SACCHARINA* WALKER (LEPIDOPTERA:PYRALIDAE), ITS HOST PLANTS AND NATURAL ENEMIES IN ETHIOPIA

2.6.1 Abstract

Surveys for sugarcane stem borers were undertaken in Ethiopia to determine the prevalence and distribution of these and their natural enemies in crops and indigenous host plants. *Eldana saccharina* was not recovered from sugarcane, but was present in three indigenous wetland sedges, *Cyperus papyrus, C. fastigiatus* and *C. dives* in the southern, central and northern part of the country. The latter indigenous host plant was present in waterways adjacent to sugarcane on the commercial sugar estates. The tachinids *Schembria eldanae* and *Actia* sp. were common parasitoids of *E. saccharina* larvae in these indigenous sedges. The braconid *Dolichogenidea* sp. was recovered from *E. saccharina* larvae in *C. dives*. Pathogens comprising *Beauveria bassiana*, *Bacillus thuringiensis* and *Entomophthora* sp., were recorded infecting *E. saccharina* larvae in the indigenous sedges. This section reports the occurrence of *E. saccharina* in Ethiopia for the first time, and it records the host plant preferences of the borer and its indigenous natural enemies found during the surveys. In addition, its potential threat to sugarcane production in Ethiopia is discussed.

2.6.2 Introduction

Eldana saccharina Walker (Lepidoptera: Pyralidae), is indigenous to Africa and surrounding islands where it feeds on a variety of host plants (Carnegie 1974; Betbeder-Matibet 1981; Conlong 1997, 2001; Polaszek and Kahn 1998; Mazodze and Conlong 2003). Wetland sedges (Cyperaceae, Juncaceae, Typhaceae) make up a large proportion of its natural host plants (Atkinson 1980; Conlong 2001; Mazodze and Conlong 2003) and it also occurs in a number of grasses (Poaceae) (Betbeder-Matibet 1981; Maes 1998). The borer was first described from sugarcane in Sierra Leone over 100 years ago (Walker 1865). Since then it has been reported throughout much of sub-Saharan Africa (Girling 1972; Waiyaki 1974; Atkinson 1980; Betbeder-Matibet 1981; Maes 1998). In southern Africa, *E. saccharina* was collected from Mozambique in 1903 and from South Africa in 1928, but the insect was first noticed as a pest of sugarcane in 1939 when an outbreak occurred on mature sugarcane in South Africa (Dick 1945). Since 1970, however, *E. saccharina* has been of major concern to the South African sugar industry and it is now a serious pest over much of the sugarcane growing regions of South Africa (Paxton 1982; Webster *et al.* 2005). In Zimbabwe, where the pest had only been encountered in sedges prior to 1998, a severe outbreak on sugarcane was reported in 1998 (Mazodze *et al.* 1999), from where it has spread to other estates in the area (Mazodze and Conlong 2003).

The trend in East Africa is similar to what was observed in Southern Africa. In East Africa, *E. saccharina* was collected in 1900 in Tanzania and in 1931 in Kenya but heavy infestation by the pest was recorded only in 1966 in a sugarcane estate in Tanzania (Waiyaki 1974). In repeated surveys conducted in Uganda from 1965 to 1968, *E. saccharina* was recovered from maize, sorghum, sugarcane and wild sedges at scattered

points, but it was not regarded as a serious pest at that time (Girling 1972). However, in 1970 it caused serious damage to sugarcane in some parts of Uganda (Girling 1972), and is still considered a pest in sugarcane in western Uganda (Conlong and Mugalula 2001).

Currently, this insect is an important pest of graminaceous crops in many widely separated parts of Africa (Conlong 1997; Bosque-Perez and Schulthess 1998; Seshu Reddy 1998). These records show that *E. saccharina* is spreading as a crop pest throughout sub-Saharan Africa. It is thus important that baseline biological surveys in crop and indigenous host plants be conducted to assess the status of *E. saccharina* and its natural enemy complex in Ethiopia, to determine whether it threatens sugarcane production in the country, and if it is amenable to control through habitat manipulation (Conlong and Kasl 2000) and/or cultural control (Carnegie 1974).

This section presents results of exploratory surveys for *E. saccharina,* its host plant complex and its parasitoids in sugarcane growing areas of Ethiopia.

2.6.3 Materials and Methods

2.6.3.1 Survey sites

Surveys were completed in the three sugarcane estates (Finchawa, Metehara and Wonji) and in peasant farms in the three major sugarcane-growing regions of Oromia, Amhara and Southern Nations Nationalities and Peoples' Region (SNNPR). Description of the survey areas is given in section 2.2.1 of this chapter.
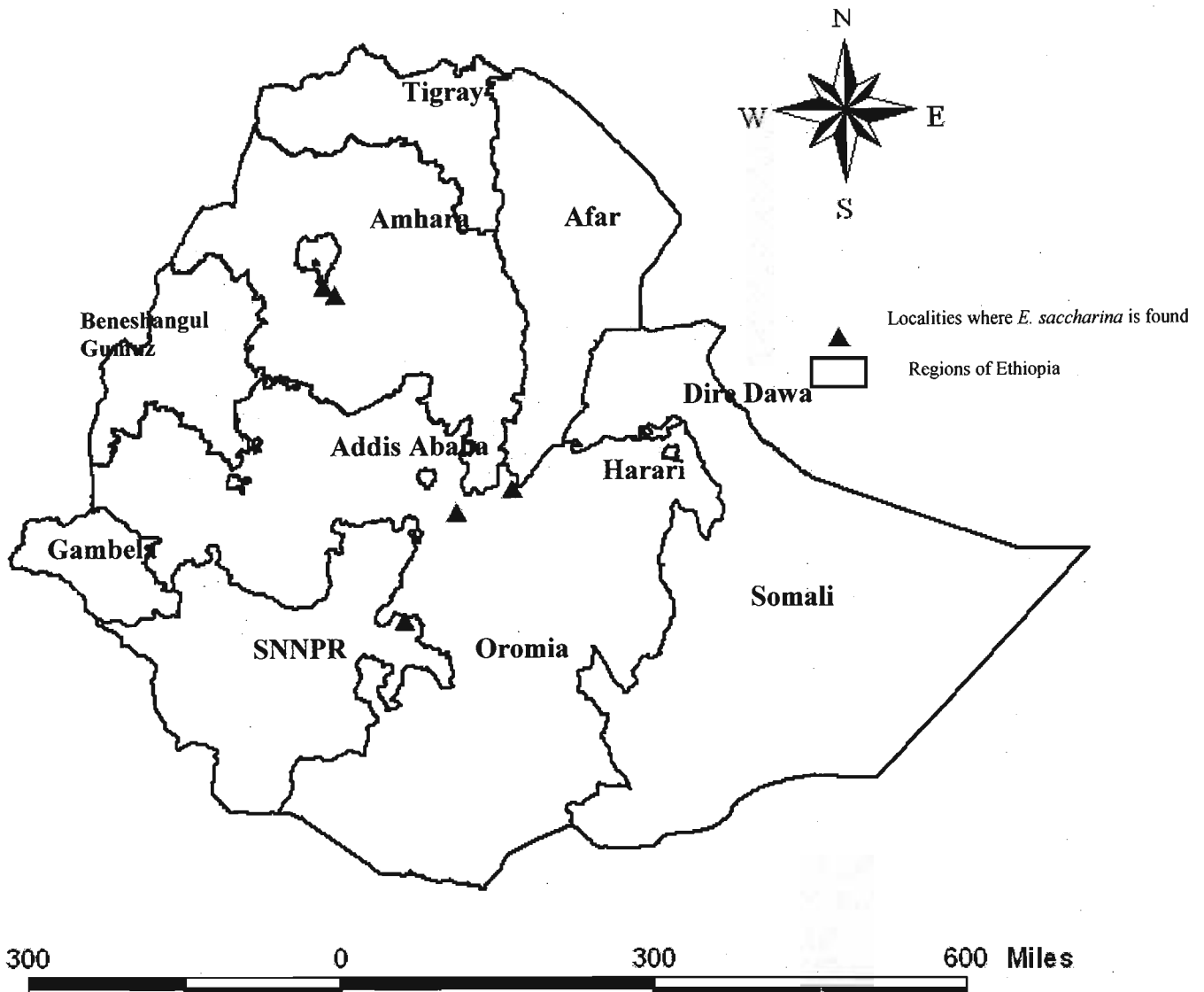
Figure 2.6   Map of Ethiopia showing localities where indigenous host plants were found

infested by *Eldana saccharina*. Names of localities are indicated in Table

2.11. Regions of Ethiopia are named.

2.6.3.2 Survey methods

Details of the survey methods are described in section 2.2.2 of this chapter.

2.6.4 Results

2.6.4.1 Eldana saccharina *in indigenous host plants*

Indigenous host plants attacked by *E. saccharina* and damage intensities observed in the surveys are shown in Table 2.11. *E. saccharina* was recorded from three wetland sedges. At Metehara (08°49′N; 39°58′E) and Wonji (8°31′N; 39°12′E) it was collected from *Cyperus dives* C.B.Cl. (Cyperaceae). In wetlands at Betemengist Sefera (11°29′N; 37°33′E) bordering the Nile River, *E. saccharina* was collected from *C. dives, C. papyrus* L. and *C. fastigiatus* Rottb. (Cyperaceae), while at Lake Awasa (07°03′N and 38°28′E) it was in *C. dives* and at Lake Tana (11°35′N; 37°23′E) it was found in *C. papyrus* (Figure 2.6). Wild grasses in farm fields and wetlands were examined for infestation by the borer, but none of these were attacked by *E. saccharina*.

In the sedges attacked, larvae and pupae of *E. saccharina* were all recovered from the umbels of mature plants. Rhizomes not covered with water were checked for infestation but no boring or life stage of *E. saccharina* was found. The infestation in sedges by *E. saccharina* ranged from 17.5% of umbels in *C. dives* at Metehara estate, Central Ethiopia, to 100 % of *C. dives* umbels searched in Lake Awasa, Southern Ethiopia.

2.6.4.2   Eldana saccharina *in sugarcane fields*

Forty-five peasant sugarcane fields and 174 sugarcane plots in the three estates were inspected and all were free of infestation by *E. saccharina*. Stalk borings were, however, found in most of the fields, but no *E. saccharina* life stages were collected from them.

2.6.4.3 *Natural enemies of* E. saccharina

In surveys done of indigenous host plants, three parasitoid and three pathogen species were recovered from *E. saccharina* larvae found in the umbels of *C. papyrus, C. dives* and *C. fastigiatus* (Table. 2.12). *Schembria eldanae* Barraclough (Diptera: Tachinidae) and *Actia* sp. (Diptera: Tachinidae) were common larval parasitoids in *C. papyrus* umbels, with parasitism levels of 5.26% and 6.33% respectively, being recorded at the time of surveys.

Table 2.11   Indigenous host plants examined for *Eldana saccharina* in the surveys conducted during 2003 and 2004 in various regions of Ethiopia, number of life stages found and infestation levels. Names in bold are names of the regions.

Locality	Position	Alt. (m.a.s.l.)	Host plant	Part of the plant	No of borers found	% Infestation
Amhara						
Betemengist Sefera	11°29′N; 37°33′E	1705	*C. dives*	Umbel	3	52
			C. papyrus	Umbel	38	68
			C. fastigiatus	Umbel	6	75.8
Lake Tana	11°35′N; 37°23′E	1700	*C. papyrus*	Umbel	79	44.1
Mankusa	10°40′N; 37°11′E	1850	*P. purpureum*	Stalk	0	0
Oromia						
Wonji estate	08°31′N; 39°12′E	1500	*C. dives*	Umbel	11	Not available
			T. latifolius	AGP	0	0
Metehara estate	08°49′N; 39°58′E	960	*C. dives*	Umbel	3	17.5
Finchawa	09°52′N; 37°19′E	1635	*S. arundinaceum*	Stalk	0	0
Ziway	07°55'N; 38°43'E	1647	*T. latifolius*	AGP	0	0
			P. purpureum	Stalk	0	10
SNNPR						
Lake Awasa	07°03′N; 38°28′E	1685	*C. dives*	Umbel	36	100

AGP, Above Ground Part.

The bacterium, *Bacillus thuringiensis* Berliner (Eubacteriales: Bacilliaceae), and a fungus, *Entomophthora* sp. (Entomophthorales: Entomophthoraceae), were also found attacking *E. saccharina* in *C. papyrus* umbels, with percentage parasitism of 10.13% and 5.26% respectively. A solitary braconid, *Dolichogenidea* sp. (Hymenoptera: Braconidae) was recovered from *E. saccharina* larvae in *C. dives* in Lake Awasa and the fungus *Beauveria bassiana* Balls. (Deuteromycotina: Hyphomycetes), was recovered from *E. saccharina* larvae in *C. papyrus* in Lake Tana. However, the percentage infestation by these natural enemies at the time of the surveys was relatively low, at 2.78 and 1.27% respectively.

Table 2.12 Natural enemies of *Eldana saccharina* recorded in the surveys conducted during 2003 and 2004 from indigenous sedges in various parts of Ethiopia. Names in bold are names of the regions.

Location	Position	Alt. (m.a.s.l.)	Natural enemy found Species	No.	Life stage attacked	Host plant	% Par.
Amhara							
Betemengist Sefera	11°29′N; 37°33′E	1705	*Schembria eldanae*	2	Larvae	*C. papyrus*	5.26
			Entomophthora sp.	2	Larvae		5.26
			Entomophthora sp.	1	Larvae	*C. fastigiatus*	16.6
Lake Tana	11°35′N;37°23′E	1700	*S. eldanae*	4	Larvae	*C. papyrus*	5.06
			Entomophthora sp.	3	Larvae		3.8
			Beauveria bassiana	1	Larvae		1.27
			Baccilus thuringiensis	8	Larvae		10.13
			Actia sp.	5	Larvae		6.33
SNNPR							
Lake Awasa	07°03′N;38°28′E	1685	*Dolichogenidea* sp.	1	Larvae	*C. dives*	2.78

2.6.5 Discussion

2.6.5.1 Eldana saccharina *in indigenous host plants*

Previous studies on biological control agents in southern Africa have shown that there are high levels of parasitism of *E. saccharina* in indigenous hosts, while very little is found in cultivated sugarcane (Conlong 1990; Conlong 1994). Similar results were obtained from surveys in Kenya (Conlong 2000) and Uganda (Conlong and Mugalula 2001). This study expands the host plant and natural enemy distribution knowledge of *E. saccharina* to relevant areas in Ethiopia, and provides the first records of host plant-*E. saccharina*-natural enemy interactions in this country. As a result, three different parasitoids, and three pathogen species were found attacking larvae of *E. saccharina* in umbels of *C. papyrus*, *C. dives* and *C. fastigiatus*. It appears that these wetland sedges are the dominant hosts of *E. saccharina* in Ethiopia, and more importantly, also the habitat of very effective natural enemies of *E. saccharina* within them (Conlong 1990). The same species of sedges were recorded to be the predominant hosts of this pest in other parts of Africa (Girling 1972; Atkinson 1979; Atkinson 1980; Mazodze and Conlong 2003), especially in the southern and eastern regions (Conlong 2001). In contrast, wild grasses, *Pennisetum purpureum* Moench.(Poaceae) and *Sorghum arundinaceum* (Desv.) Stapf. (Poaceae) that were reported to host *E. saccharina* in West Africa (Girling 1972; Betbeder-Matibet 1981; Maes 1998; Polaszek and Khan 1998; Conlong 2001) were free from the pest in Ethiopia.

It is important to have a detailed knowledge and proper understanding of the potential hosts of stem borers to effectively prevent or reduce damage in graminaceous crops (Polaszek and Khan 1998). Studies on the ecology and natural enemy complex in

indigenous host plants will provide an insight into the type of natural enemies to use in managing the pest in crop plants (Conlong 1997), and in developing habitat management strategies (Conlong and Kasl 2001). This study has provided evidence that *E. saccharina* occurs in certain species of sedges in Ethiopia, and that there are indigenous parasitoids attacking its life stages in these host plants. However, for a scientifically sound incursion plan to be developed to prevent possible infestation of sugarcane by *E. saccharina*, there is a need to conduct a more detailed study on the diversity of indigenous host plants of *E. saccharina* in Ethiopia and the population dynamics over time of the pest and its natural enemies in these indigenous habitats.

2.6.5.2 Eldana saccharina *in sugarcane*

Available reports from other African countries suggest that *E. saccharina* is an indigenous pest that in most countries has established in sugarcane and other introduced graminaceous crops in which it thrives (Polaszek 1998). Repeated area wide surveys on lepidopterous stem borers of maize and sorghum conducted in Ethiopia (Gebre-Amlak 1985; Getu *et al.* 2001; Tefera 2004) have shown that *E. saccharina* is not amongst the complex of stem borers attacking these grain crops. This study shows that this is also the case for Ethiopian sugarcane, even though *E. saccharina* is the major borer in umbels of large sedges growing in irrigation channels in sugarcane estates and along rivers and lake banks amongst peasant sugarcane fields. Whether or not it will spread into sugarcane and other cereal crops remains to be seen, but the possibility of it invading sugarcane certainly exists, as recent history shows. In Zimbabwe, where the borer was first observed in sedges close to sugarcane in 1987, a severe outbreak in sugarcane by *E. saccharina* was reported from two fields in 1998 (during a severe drought), and has since then spread throughout their

industry (Mazodze *et al*. 1999; Mazodze and Conlong, 2003). The same may happen in Ethiopia should current biotic and/or abiotic factors change to favor the incursion of *E. saccharina* into sugarcane. Climate and sugarcane expansion and related agronomic factors should continually be monitored in order to predict the relevant changes, and to take corrective action before serious infestation occurs.

As sugarcane production in Ethiopia is dependant on furrow irrigation from springs and rivers, it is not unusual to see small-scale sugarcane fields located in or nearby swampy areas. The biggest sugarcane estates, Metehara and Wonji, are also established on the banks of the Awash River, which is their sole source of water for irrigation. These swamps, channels and riverbanks are natural habitats of sedges from which *E. saccharina* was collected. Should encroachment of the crop into indigenous host plant habitats (which also harbour natural enemies) of the insect take place, then there is a real danger of the insect moving into the crop, because of reduced natural enemy numbers because of reduced habitat, as hypothesized by Conlong (1997).

This move can be further exacerbated by having over-aged cane left standing in the field. In southern Africa and Uganda it has been clearly demonstrated that *E. saccharina* prefers older sugarcane (Nuss *et al*. 1986; Conlong and Mugalula 2001). In the Ethiopian estates sugarcane is left in the field before harvest for up to 22 months and in small-scale farms it will be harvested only when there is a market for it. These practices make the plants vulnerable to *E. saccharina* attack. The sugar estates and small-scale farmers in Ethiopia should be mindful of this fact, and manage their harvesting accordingly; to minimize the chance of *E. saccharina* colonization and population build up.

One of the reasons that *E. saccharina* prefers older sugarcane is because in these older plants, nutrients are no longer used for plant growth, especially nitrogen (Nuss *et al.* 1986), which then becomes available for insect use. It has also been shown that *E. saccharina* infestations increase as nitrogen fertilizer application rates increases (Carnegie 1981). Reduction of nitrogen fertilizer to 30kg per hectare is recommended to reduce *E. saccharina* problems in South African sugarcane (SASA 1994). Ethiopian sugar estates, however, practice the blanket application of high amounts of fertilizer, 200 to 700kg/ha of Ammonium Sulphate Nitrate (ASN), which contains 26% nitrogen (Kedru 1993). This additional nitrogen, which the sugarcane plant cannot use, will certainly increase the chance of colonization, survival and growth of *E. saccharina*. Careful monitoring with regards to the application of high nitrogen fertilizers should be practiced, so that only the amount needed by the plant is applied, leaving no available nitrogen in the plant that can be exploited by the insect.

In South Africa, sugarcane varieties show different levels of resistance to *E. saccharina*. (Nuss *et al.* 1986; Keeping and Rutherford 2004). The number of internodes bored, larval mass and *E. saccharina* population density was found to vary between varieties (Nuss *et al.* 1986; Rutherford *et al.* 1993; Bond 1988; Keeping 1999). Thus, it is advisable to regularly monitor sugarcane fields adjacent to water bodies for infestation by *E. saccharina* and to avoid planting varieties showing *E. saccharina* susceptibility in fields bordering indigenous host plant stands to minimize the chance of colonization by the borer.

2.6.5.3 *Natural enemies of* E. saccharina

The present surveys in Ethiopia revealed a number of parasitoids and pathogens attacking *E. saccharina* in its indigenous host plants. As there was no available information on *E.*

saccharina in Ethiopia, the natural enemies recorded in these surveys are all new records for the country. The tachinids *S. eldanae* and *Actia* sp. were the common larval parasitoids in *C. papyrus* umbels. The former larval parasitoid was described by Barraclough (1991) from *E. saccharina* in *C. papyrus* in KwaZulu-Natal, South Africa. Currently *S. eldanae* is known as a parasitoid of *E. saccharina* on *C. papyrus* in few localities in South Africa (Harris 1998), Kenya (Conlong 2000) and Uganda (Conlong and Mugalula 2001). *Actia* spp. are reported to be parasitoids of the important stem borer species in Sierra Leone, Cameroon and Uganda (Harris 1998). However, there was no information available on the existence of these parasitoids in eastern Africa (Conlong 2001). The other parasitoid recovered in these surveys is the solitary braconid, *Dolichogenidea* sp., from *E. saccharina* larva in *C. dives* at Lake Awasa. Parasitoids in this genus are known for their long ovipositor, used to reach concealed living hosts, and are reported from important stem borers (van Achterberg and Walker 1998). Recently, *Dolichogenidea fuscivora* Walker (Hymenoptera: Braconidae) was reported from *Busseola fusca* Fuller (Lepidoptera: Noctuidae) in eastern Ethiopia (Tefera 2004). The record of this parasitoid from *E. saccharina* in the current survey in the southern part of the country indicates a wide distribution and host range of *Dolichogenidea* spp. This merits further study of this parasitoid group as biocontrol agents in the management of stem borers in Ethiopia.

Similarly, emphasis needs to be given to the bacterial and fungal pathogens that are common mortality factors of *E. saccharina* in its indigenous sedge host plants. The conspicuous symptoms of *Entomophthora* sp., seen occasionally in infected *E. saccharina* larvae in South Africa (Carnegie 1987), were more frequent in *C. papyrus* umbels in Lake Tana. In addition, the impact of the *B. thuringiensis* isolate from Ethiopia needs to be studied. Jacobs (1989) in South Africa, found some *B. thuringiensis* isolates to be highly

toxic to *E. saccharina* larvae. Also, *B. bassiana* was reported to attack *E. saccharina* both in its natural habitat and in cultivated crops in South and West Africa (Conlong 1990, 2001). The presence of *B. bassiana* in Ethiopia shows the wide adaptation of the pathogen in diversified habitats.

It is evident that in its indigenous host plants in Ethiopia, *E. saccharina* has a complex of indigenous natural enemies keeping it in check. This is in keeping with similar surveys conducted in other African countries, where rich natural enemy guilds have been discovered (Conlong 2000, 2001). Of particular interest is the presence of the tachinids *S. eldanae* and *Actia* spp. In previous surveys, the former has always been a component of the parasitoid guild attacking *E. saccharina* in eastern and southern Africa (Conlong 2001), while the latter has formed part of the guild attacking *E. saccharina* in western Africa (Conlong 2001). In a review on biocontrol of *E. saccharina*, Conlong (2001) associated the very different parasitoid fauna collected from *E. saccharina* in West Africa, compared to East and southern African regions, to possible biotypical differences in *E. saccharina* populations. This view was further supported by the results of molecular analyses on populations of *E. saccharina* from the different parts of Africa (King *et al*. 2002; Assefa *et al*. 2005). Occurrence of parasitoid faunas from different regions of the continent in Ethiopia, therefore, could be related with the existence of different biotypes of *E. saccharina* in this region and merits further study.

These surveys highlight the existence of a rich natural enemy guild that is keeping the pest in check in its natural habitat. However, identification of the natural enemy complex existing in the country and the pest-parasitoid relationships in the natural habitat requires regular and extensive surveys of the natural host plants of *E. saccharina*. Such studies were

found to reveal the key natural enemies that keep *E. saccharina* in check in its natural habitat in South Africa (Conlong 1990) so that they could be collected and tested against this borer in its crop hosts.

2.6.6 Conclusions

This chapter provides the first evidence that *E. saccharina* is not attacking sugarcane in Ethiopia, but that it is present in the indigenous sedges *C. papyrus, C. dives* and *C. fastigiatus* throughout the Ethiopian sugarcane growing regions. In addition, it provides the first evidence that these indigenous sedge species house a complex of natural enemies that attack *E. saccharina*. These natural enemies have been found previously in other African countries but their known distribution is now expanded into Ethiopia. The complex comprises the insect parasitoids, *S. eldanae, Actia* spp. and *Dolichogenidea* sp., and the pathogens, *B. thuringiensis, B. bassiana* and *Entomophthora* sp., all of which attack the larval stages of *E. saccharina*. These natural enemies limit *E. saccharina* population growth in these plants, which may makes this indigenous host plant habitat very useful in preventing *E. saccharina* incursions into sugarcane.

Sugarcane farmers in Ethiopia should be aware that *E. saccharina* has moved from natural hosts to sugarcane in South Africa, Zimbabwe, Uganda and Kenya. Factors preventing it from moving into sugarcane fields in Ethiopia need to be studied and preventative methods followed. Agronomic practices that tend to increase *E. saccharina* populations in sugarcane need to be discouraged, especially in times of drought. The diversity of natural enemies in the country need to be further investigated in time and space, and the natural

enemies in their indigenous habitat should be evaluated for their role in the management of *E. saccharina* in sugarcane in other parts of Africa.

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CHAPTER 3

MOLECULAR PHYLOGENY AND PHYLOGEOGRAPHY

3.1 PHYLOGEOGRAPHY AND PHYLOGENY: AN OVERVIEW

3.1.1 Phylogeography

Phylogeography concerns the principles and processes determining the geographical distribution of genetic lineages, especially those within and amongst closely related species (Avise 2000). It is useful in understanding processes such as population subdivision, speciation and ecological adaptation to past climatic changes (Avise 1998). Phylogeography started as a formal discipline in the late 1980s (Avise *et al.* 1987), although the field's conception began in the mid 1970s with the introduction of mitochondrial DNA (mtDNA) analyses to population genetics, and the profound shift towards a genealogical perspective at the intraspecific level (now formalized as coalescent theory) that these methods prompted (Avise 1998). Phylogeography integrates molecular genetics, population genetics, phylogenetics, demography, ecology and historical biogeography. Emphasis is put on historical aspects of the contemporary distribution of gene genealogies. In its purest form, empirical phylogeographic analysis deals with the special distributions within and amongst populations of alleles whose phylogenetic relationships are deduced (Avise 1998).

3.1.2 Phylogeny

The study of phylogeny began before the turn of the century, even before Mendels's laws

were rediscovered in 1900 (Graur and Li 1999). The hierarchical system of nomenclature developed by Linneaeus was initially independent of evolutionary theory, and in fact early evolutionists opposed the Linnaean system and favoured Aristotelian essentialism (Moritz and Hillis 1996). However, the Linnaean system prevailed and later evolutionists simply co-opted the system to produce classifications based on phylogenetic relationships. Initial efforts to reconstruct phylogenetic history were based on few objective criteria, and estimates of phylogeny were little more than plausible assertions by experts on particular taxonomic groups. The situation began to change during the 1930s-1960s through the efforts of scientists that began to define objective methods for reconstructing evolutionary history based on shared attributes of extant and fossil organisms (Moritz and Hillis 1996).

Since the late 1960s, various techniques have been developed in molecular biology, which started the extensive use of molecular data in phylogenetic research (Graur and Li 1999). In particular, the study of molecular phylogeny progressed tremendously between the late 1960s and 1970s as a result of the development of protein sequencing methodologies and new analytical methods. Less expensive and more expedient methods such as protein electrophoresis, DNA-DNA hybridisation, and immunological methods, though less accurate than protein sequencing, were extensively used to study the phylogenetic relationships among populations or closely related species (Hillis *et al.* 1996; Collins *et al.* 1988). The application of these methods also stimulated the development of measures of genetic distance and tree making methods (Nei 1975; Swofford 2002).

The rapid accumulation of DNA sequence data since the late 1980s due to the advent of various molecular techniques, particularly polymerase chain reaction (PCR), has resulted in an unprecedented level of activity in the field of molecular phylogenetics. These data are

widely used to infer phylogenetic relationships among closely related populations or species (Brower 1994; Danforth *et al.* 1998; Evans *et al.* 2000) and phylogeographic structures within species (Vandewoestijne *et al.* 2004).

3.1.3 Review of Molecular Techniques in Phylogenetic Studies

Phylogenetic and phylogeographic research is facilitated by methods that reveal genetic variation. There exist wide varieties of methods to reveal genetic variation (Avise 1994). Protein electrophoresis is one method to study genetic variation (Murphy *et al.* 1996). DNA analysis is also used to estimate genetic variation within and among populations (Dowling *et al.* 1990). Restriction Fragment Length Polymorphism (RFLP) in mitochondrial and unique nuclear sequences has provided useful genetic markers for the analysis of variation within species. However, as technology becomes more accessible, most researchers are abandoning RFLP studies in favour of approaches that determine DNA sequences directly. Applications are broad and include estimating the extent of variation within and between populations, levels of gene flow and analysis of parentage and relatedness (Dowling *et al.* 1996). Each of these methods has specific areas of application for phylogenetic and phylogeographic studies.

3.1.3.1 Protein electrophoresis

Protein electrophoresis takes advantage of the fact that nondenatured proteins with different net charges migrate at different rates through a support medium under the influence of an electrical field. It is the most cost-effective method for investigating genetic variation at the molecular level (Murphy *et al.* 1996). Tissue samples are homogenized;

run on the gel matrix to achieve sufficient protein separation, and histochemically stained to visualize the specific protein in question. Detectable proteins include functionally similar forms of enzymes designated as isozymes (Murphy *et al.* 1990) and allozymes, which are variants of polypeptides representing different allelic alternatives of the same gene locus (Murphy *et al.* 1996).

Nondenatured proteins migrate at different rates through starch or acrylamide gels to which an electric current is applied (Avise 1994). The net charge of a protein, which varies with the pH of the running condition, determines the protein's movement in an electrical field (Avise 1994). Protein size and shape also can interact with pore size in the electrophoretic matrix to influence migrational properties (Avise 1994). Most of the common amino acids are neutrally charged except lysine, arginine and histidine which have positive side chains, and aspartic and glutamic acid that are negatively charged (Avise 1994). Only substitutions involving these amino acids are detectable by protein electrophoresis. Size may vary because of nucleotide re-arrangements resulting in restructure of protein amino acids.

Although electrophoresis reveals genetic differences, loss of information may result from non-detectable (neutral) differences, e.g., replacement of an amino acid with one that has the same net charge (Avise 1994). In addition, it is estimated that no more than one third of all nucleotide changes cause amino acid changes – the remainder are "silent" substitutions, not detectable by protein electrophoresis (Avise 1994).

Allozymes are ideal genetic markers for assessing parentage and gene flow (Avise 1994). Assessment of allozyme variability may also be used to infer historical events that have significantly influenced the genetic structure of populations (Murphy *et al.* 1996). Studies

on several species of introduced birds and on amphibians evidenced that allozyme data are suitable to estimate effective population size and founder effect (Murphy *et al.* 1996).

3.1.3.2 DNA-DNA hybridization

The DNA-DNA hybridization method relies on the double-stranded nature of duplex DNA in which the two complementary strands are held together by weak hydrogen bonds (Werman *et al.* 1996). These hydrogen bonds are the weakest links in the DNA, so when native DNA is heated, the hydrogen bond between complementary base pairs are broken and opposing strands separate. As the heated sample is cooled, stands collide by chance, and those with complementary nucleotide sequence re-associate into double-stranded molecules as their respective bases pair and reform hydrogen bonds.

DNA strands from two different species can be mixed under conditions where duplex formation occurs. The double stranded molecules that form between complementary strands from the two species will contain base pair mismatches because of the evolutionary divergence between the species. The thermal stability exhibited by the hybrid molecule depends largely on the extent of base pair mismatches in its two strands. The measured difference in thermal stability between homoduplexes and heteroduplexes provides a quantitative estimate of the genetic divergence between the two species (Avise 1994).

DNA hybridization data have sometimes been promoted as the strongest available source of phylogenetic information as they involve an averaging of genetic differences across a large fraction of the genome (Avise 1994). DNA-DNA hybridization approaches have had tremendous impact in molecular genetics by revealing important aspects of genomic

structure, such as the amounts of repetitive DNA, length of repeated sequences, and interspersion patterns among repetitive and low-copy sequences. With regard to phylogenetic applications, reservations expressed about the DNA hybridization approach include the fact that the raw data consist solely of distance values and that the influences of the factors affecting the kinetics of hybridization are incompletely understood (Avise 1994). DNA hybridization estimates the amount of sequence divergence between genomes, but cannot provide discrete character data and does not resolve the nature of the sequence variation (Dowling *et al.* 1996).

3.1.3.3 DNA sequencing and polymerase chain reaction

Nucleic acid sequencing is a comparatively new approach for systematics. This technology has developed rapidly over the past two decades to become the most utilized of the molecular approaches for inferring phylogenetic history (Hillis *et al.* 1996). Nucleotides are used as character states in DNA sequencing analysis. Silent and neutral substitutions undetected by allozyme analysis are detectable by DNA sequencing (Hillis *et al.* 1990). Selected regions of the genome are sequenced for each individual and aligned. Nucleotide site changes can be converted into quantitative measurements of genetic distance or each nucleotide can be treated as a discrete character state.

In the past, nucleic acid sequencing was limited by the availability of purified homologous DNA from different organisms. Such sequences had to be isolated and amplified in vivo by laborious procedures of cloning into microbial vectors (Avise 1994). The development of polymerase chain reaction (PCR) (Mullis and Faloona 1987) has changed this situation dramatically by permitting rapid in vitro DNA amplifications. The PCR technique uses a

thermostable bacterial DNA polymerase, *Taq*, to replicate DNA. Repeated cycling of the replication event produces millions of copies of a specific genomic region. PCR reduces the amount of DNA required, and specific primers permit amplification of different regions of the genome. As a result, DNA sequencing has grown explosively and has become the most popular source of data for phylogenetic reconstruction (Avise 1994).

The PCR technique involves three steps: DNA template denaturation, primer annealing and primer extension. In the denaturation step, heat is used to stop all enzymatic reactions and denature the DNA from double to a single strand. The temperature used in the denaturation process is usually 94°C for 30 seconds, though it varies from protocol to protocol (Palumbi 1996). Too high a temperature will reduce enzyme activity and too low a temperature will result in incomplete denaturation. The annealing phase is the most critical phase. In this phase the temperature is lowered to enable oligonucleotide primers to bind to the appropriate sites on the template DNA. If the temperature is too high not enough primer is bound, but if it is too low then multiple annealing will occur and artifacts are generated. Shorter annealing times seem to provide greater specificity in the PCR reaction than longer ones. Generally annealing times of 30-60 seconds are most common, although times as short as 15 seconds often work well at high annealing temperatures with perfect primers (Palumbi 1996). The extension phase allows the enzyme to synthesize the target DNA segment. *Taq* polymerase works well at about 72°C, and this is the temperature usually chosen for extension. The extension time varies based on the size of the PCR product. As a rule of thumb one minute is sufficient for every 1 000 bp of the expected size of the PCR fragment. However, optimization may be required because an unnecessarily long extension time can increase the likelihood of PCR artefacts (Avise 1994).

3.1.3.4 Restriction Fragment Length Polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) analyses use restriction enzymes to cut DNA at specific oligonucleotide sequences, usually four, five or six base pairs in length (Avise 1994). Many such enzymes have been isolated and characterized from various bacterial strains. Sample DNA is cut (digested) with one or more restriction enzymes and resulting fragments are separated according to molecular size using gel electrophoresis (Avise 1994). Ethidium bromide staining is used to visualize the fragments under ultraviolet light. Molecular size standards are used to estimate fragment size. Differences result from base substitutions, insertions or deletions within restriction enzymes recognition sequences, or sequence rearrangements (Avise 1994).

Although fragment analysis offers less resolution than nucleotide sequencing in some respects, it is a powerful and cost effective alternative where large numbers of individuals or loci or large segments of a genome are being screened (Dowling et al. 1996).

3.1.4 Molecular Markers

Most studies that utilize molecular markers can be viewed as attempts to estimate phylogeny, at one or another hierarchical stage of evolutionary divergence (Avise 1994). Phylogenetic relationships thus can be assessed at levels ranging from extreme micro to macro-evolutionary levels. Different kinds of molecular data provide genetic information ideally suited to different subsets of this hierarchy. DNA sequencing has become the method of choice for most molecular systematic studies, however it should not be forgotten that many alternatives might be more appropriate or more practical for certain applications

(Caterino *et al.* 2000). Rapid progress has been achieved in certain areas of ecology and evolutionary biology because newly available markers can identify individuals, populations, genetic strains, or closely related species (Parker *et al.* 1998). Thus it is becoming increasingly important for population biologists to be able to understand and evaluate molecular data and to know whether their own research questions could be addressed with molecular techniques.

3.1.4.1 Nuclear genome

The nuclear genome is inherited from both parents in sexually reproducing species with recombination occurring during meiosis (Futumya 1986). Most of the nuclear DNA consists of non- coding regions and heterochromatin. Protein coding regions vary from single copy loci to repetitive arrays (gene families). Translated loci can be monitored for variation using allozyme analysis (Murphy *et al.* 1990). For molecular analysis it is useful to study specific genomic regions. One region that is commonly used is the ribosomal DNA (rDNA) gene family.

The rDNA gene family is a multigene family consisting of many copies (100-500 in animals) of genes encoding for three ribosomal components; in animals these are 28S, 5.8S and 18S (Black *et al.* 1989). In eukaryotes, the 5' to 3' organization of the gene family is an external transcribed spacer (ETS); the 18S gene; an internally transcribed spacer (ITS1); the 5.8S (or 5S in plants) gene; ITS2; the 28S (or 26S in plants) gene; and the intergenic spacer (IGS) (Gerbi 1985). Subsequent to the IGS region follows another copy of the gene family.

Highly conserved regions in the ribosomal repeat array can be used for the study of relationships across Phyla (Gerbi 1985), more variable regions can be used at lower taxonomic levels. The ITS region does not encode for any product, thus it evolves at a faster rate than the ribosomal coding regions. The level of variation in this region makes it suitable for lower level phylogenetic studies.

Slowly-evolving nuclear protein-coding genes have recently become available for phylogenetic analysis in many groups of insects. These genes have a number of advantages over ribosomal genes. Most obviously, they are easily alignable. Many of these genes have been demonstrated to be capable of recovering divergences in insects (Friedlander *et al.* 2000; Wiegmann *et al.* 2000; Moulton and Wiegmann 2004). Elongation factor-1alpha (EF-1alpha) (Caterino *et al.* 2000), LW rhodopsin (Cameron and Williams 2003) and Wingless (Baker *et al.* 2001) are some of the nuclear genes that are proven to be useful for studies of higher-level phylogenetic relationships in insects.

3.1.4.2 Animal mitochondrial DNA

The animal mitochondrial genome consists of a circular DNA molecule approximately 16 kilobases long that encodes 13 proteins, 22 transfer RNAs and two ribosomal RNAs (Avise and Lansman 1983). Since these products remain in the mitochondria, selection pressures and evolution often occur independently of the nuclear genome (Moritz *et al.* 1987). The small size of the animal mitochondrial genome has facilitated sequencing it in its entirety in many taxa. Complete sequences exist for several insect species including *Apis mellifera*

L. (Hymenoptera: Aphidae) (Crozier and Crozier 1993), and *Drosophila yakuba* Burla (Diptera: Drosophilidae) (Clary and Wolstenholme 1985). Besides size, the structure and genetic basis of inheritance (maternal) of mitochondrial DNA have made it better understood than any similarly sized region of the nuclear genome (Avise 1994). Mitochondrial genes are generally considered too rapidly evolving for these deep divergences and show substitution patterns that are problematic for reconstructing ancient divergences (Lin and Danforth 2004).

3.1.5   Recent Advances and Use in African Stem Borer Ecology

The number of phylogenetic and phylogeographical studies on animals has increased greatly during recent years, particularly in Europe, but are mainly concerned with vertebrate taxa (Avise 2000; Nieberding *et al.* 2005). However invertebrate taxa, particularly in Africa, still remain understudied. Currently, few phylogeographic studies on lepidopterous stem borers are being undertaken. Sezonlin *et al.* (2005) described the population structure of *Busseola fusca* Fuller (Lepidoptera: Noctuidae) and studies on other noctuid stem borers such as *Manga spp.* Bowden (Lepidoptera) (Moyal and Le Rü 2006) and *Busseola phaia* Bowden (Lepidoptera: Noctuidae) (Sylvain unpublished) are in progress. Phylogeographic studies on Crambid borers (Lepidoptera) (Mitchell unpublished) are also underway.

In this chapter, the phylogeography of *Eldana saccharina* Walker (Lepidoptera: Pyralidae) is described. It provides the first steps towards understanding the continental population structure of an important insect pest of poaceous crops in Africa. Through the results presented in this chapter, the benefits of such an approach was shown to lead to a better understanding of behavioural and population traits of different populations of insect pests,

which in turn allows more sound and scientific insect management systems to be planned and implemented.

This chapter also shows how such techniques could be used to distinguish between two species of sugarcane borers occurring in Ethiopia (Chapter 3.3), which helped explain different biocontrol assemblages found between different crops. Finally it shows how such techniques could be used to determine the origin of introduced species, as was done with *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) found on *Chilo partellus* Swinhoe (Lepidoptera:Crambidae) in Ethiopia (Chapter 3.4).

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3.2 PHYLOGEOGRAPHY OF *ELDANA SACCHARINA* WALKER (LEPIDOPTERA: PYRALIDAE)

3.2.1 Abstract

This study establishes the continental phlyogeographical pattern in Africa of the indigenous moth *Eldana saccharina* Walker. Populations of *E. saccharina* from 11 African countries were studied. A five hundred and two base pair fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene was sequenced to clarify phylogenetic relationships among geographically isolated populations from East, North, South and West Africa. The results revealed that *E. saccharina* populations are separated into four major units corresponding to the West Africa, Rift Valley, South/East Africa and southern African populations. Mitochondrial DNA divergence among the four populations ranged from 1% to 4.98%. The molecular data generally are congruent with isolation by distance pattern although some of the specimens from geographically close populations in eastern and southern Africa are genetically distant from each other. Geographical features such as the Rift Valley and large water bodies seem to have had a considerable impact on the distribution of genetic diversity in *E. saccharina*.

3.2.2 Introduction

Lepidopteran stem borers are generally considered to be the most injurious insect pests of cereals and sugarcane in sub-Saharan Africa (Polaszek 1998; Kfir *et al.* 2002). Leslie (2004) lists five species of economic importance in African sugarcane. However, two indigenous stem borers, *Eldana saccharina* Walker (Lepidoptera: Pyralidae) and *Sesamia*

calamistis Hampson (Lepidoptera: Noctuidae), are considered to be the most important (Polaszek and Khan 1998). *Eldana saccharina* is a key pest of sugarcane in western, eastern and southern Africa (Atkinson 1980; Conlong 2001). This insect has been reported from maize and sorghum in southern Africa, but seldom causes significant damage in these crops in this region (Atkinson 1980). This is in contrast to West Africa, where it is a major pest of maize and sorghum (Kaufmann 1983; Sampson and Kumar 1985; Shanower *et al.* 1993). Studies have reported that the insect exhibits considerable phenotypic and behavioral variation, displaying differential responses to control agents (Carnegie *et al.* 1985; Maes 1998; Conlong 2001; Mazodze and Conlong 2003) and preferring different host plants in various parts of Africa (Conlong 2001; Matama-Kauma *et al.* 2002; Atachi *et al.* 2005). The species' confusing behavioral patterns and diverse natural enemy guilds contrast with a lack of morphological diversity, making this insect a prime candidate for molecular systematic analysis (Maes 1998; Evans *et al.* 2000; Scheffer 2000; King *et al.* 2002). Due to the increase in the economic importance of the insect (Mazodze and Conlong 2003; Webster *et al.* 2005), there is an urgent need to control it. Ascertaining the degree of relatedness among populations is a basic prerequisite for making informed decisions regarding natural enemy selection for biological control options and correct interpretation of ecological investigations, which may be useful for habitat management control options.

Despite its high diversity and economic importance in many parts of Africa, very little is known about the population genetics and phylogeography of *E. saccharina*. The first study of the genetic structure of natural populations of *E. saccharina* and evidence of genetic variation among populations of the pest was reported by King *et al.* (2002). Later, Assefa *et al.* (2006) detailed the existence of genetic differentiation in *E. saccharina* from different

parts of Africa and suggested that geographical features play a role in limiting gene flow among populations of the pest. The East African Rift Valley has been identified as a major barrier to gene flow in vertebrates in Africa (Arctander *et al.* 1999; Pitra *et al.* 2002). Similarly, Ndemah *et al.* (2001) suggested that geographic barriers, such as mountains or forests, might facilitate the development of specific races of insect species, differing in climatic requirements and host plant specificities. Hence, it is important to evaluate the effect of geographic features on the distribution of the different populations of *E. saccharina* in the continent. The present study, therefore, builds on initial results by King *et al.* (2002) and Assefa *et al.* (2006) and investigates the impact of host plant association and geographic location on the genetic diversity of the species. The aim of this study is, therefore, to analyse the genetic structure and phylogeography of *E. saccharina* populations from different host plants in various parts of Africa.

3.2.3 Materials and Methods

3.2.3.1 Sample collection

Eldana saccharina samples used in this study were obtained from 23 localities in eleven African countries (Figure 3.1). The collection locality, date and the host plant for each specimen is indicated in Table 3.1, along with the DNA extraction number of each sequence reported in this study. Of a total of 66 specimens of *E. saccharina* used in this study, three Benin, four Ugandan and five South African specimens were included in a previous study by King *et al.* (2002) and an additional 18 specimens collected from sedges, maize and sugarcane from various localities in Ethiopia, Uganda, Senegal, Mozambique,

Zimbabwe and South Africa were reported by Assefa *et al.* (2006). The remaining 36 sequences are new.
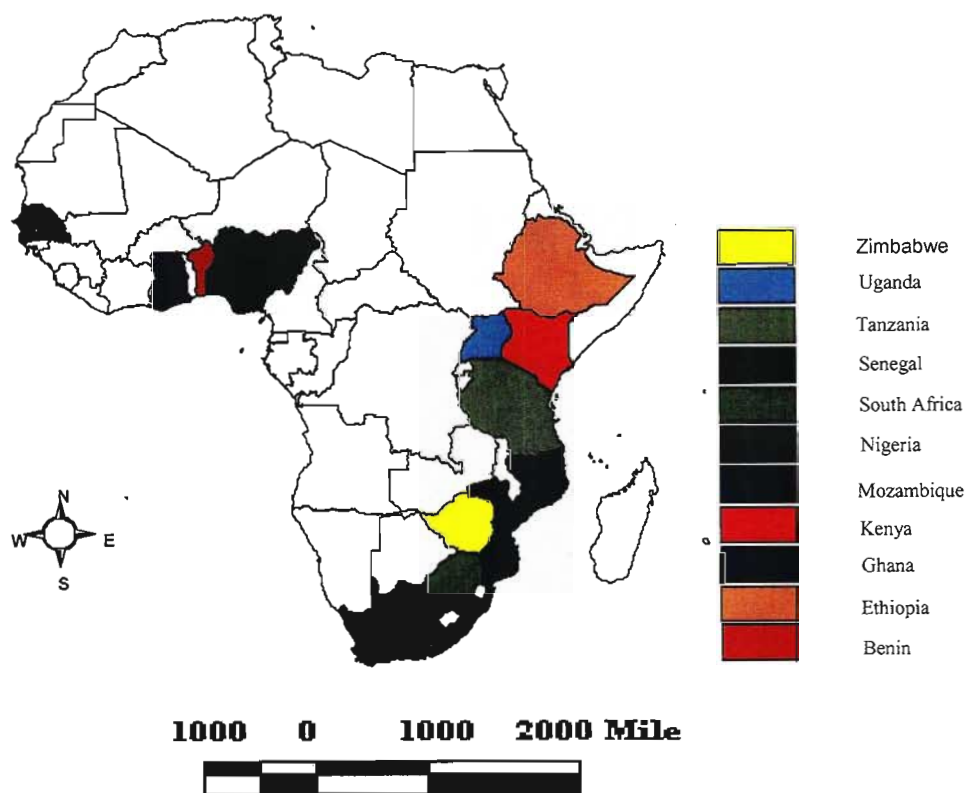
Figure 3.1   Map of Africa showing countries in which *E. saccharina* specimens were collected for the study

3.2.3.2 DNA extraction

Genomic DNA was extracted from thoracic tissue using the Qiagen DNeasy™ Tissue Kit and stored at -20°C. Voucher specimens (heads, abdomens and wings) are stored at the South African Sugarcane Research Institute (SASRI), Mount Edgecombe, KwaZulu-Natal, South Africa.

Table 3.1   African locations where specimens of *Eldana saccharina* used in the study were collected.

DNA No	Country	Location	Coordinate	Host Plant	Haplotype No
1	Benin	IITA Station, Calavi	06°25′N 02°20′E	Maize	WE Afr.-1
10	Benin	IITA Station, Calavi	06°25′N 02°20′E	Maize	WE Afr.-1
11	Benin	IITA Station, Calavi	06°25′N 02°20′E	Maize	WE Afr.-1
13	Benin	IITA Station, Calavi	06°25′N ,02°20′E	Maize	WE Afr.-2
411	Ethiopia	Lake Awasa	07°03′N 38°28′E	*Cyperus dives*	Eth-1
412	Ethiopia	Lake Tana	11°22′N 31°39′E	*C. papyrus*	Eth-2
413	Ethiopia	Lake Tana	11°22′N 31°39′E	*C. papyrus*	Eth-3
414	Ethiopia	Metehara	08°49′N 39°58′E	*C. dives*	Eth-4
742	Ghana	Twifo	?	?	WE Afr.-1
78	Kenya	Lake Naivasha	00°45′S 36°25′E	*C. papyrus*	Ken-1
85	Kenya	Lake Naivasha	00°45′S 36°25′E	*C. papyrus*	Ken-1
86	Kenya	Lake Naivasha	00°45′S 36°25′E	*C. papyrus*	Ken-1
242	Kenya	Kisumu 4	00°36′N 34°27′E	Maize	WE Afr.-1
243	Kenya	Garsen2	02°16′S 40°07′E	Maize	SE Afr.-1
88	Mozambique	Mafambisse	19°20′S 34°10′E	*C. dives*	SE Afr.-1
228	Mozambique	Marromeu	18°17′S 35°57′E	*C. papyrus*	SE Afr.-1
233	Mozambique	Marromeu	18°17′S 35°57′E	*C. papyrus*	Moz-1
271	Mozambique	Mafambisse	19°20′S 34°10′E	*C. dives*	SE Afr.-1
445	Mozambique	Marromeu	18°17′S 35°57′E	*C. papyrus*	Moz-2
446	Mozambique	Marromeu	18°17′S 35°57′E	*C. papyrus*	SE Afr.-1
447	Mozambique	Mafambisse	19°20′S 34°10′E	*C. dives*	Moz-1
448	Mozambique	Mafambisse	19°20′S 34°10′E	*C. dives*	SE Afr.-1
725	Nigeria	EGBUNA-IMO Stat	?	?	WE Afr.-1
440	Senegal	Richard Toll-c	16°25′N 15°42′W	Sugarcane	WE Afr.-2
442	Senegal	Richard Toll-c	16°25′N 15°42′W	Sugarcane	WE Afr.-2

DNA No	Country	Location	Coordinate	Host Plant	Haplotype No
444	Senegal	Richard Toll-c	16°25′N 15°42′W	Sugarcane	WE Afr.-1
4	South Africa	SASRI lab colony	29°42′S 31°02′E	N/A	SA-2
6	South Africa	SASRI lab colony	29°42′S 31°02′E	N/A	SE Afr.-1
7	South Africa	SASRI lab colony	29°42′S 31°02′E	N/A	SA-3
22	South Africa	SASRI lab colony	29°42′S 31°02′E	N/A	SE Afr.-1
23	South Africa	SASRI lab colony	29°42′S 31°02′E	N/A	SE Afr.-1
79	South Africa	Gingindhlovu West	29°02′S 31°30′E	Sugarcane	SA-1
80	South Africa	Richards Bay	28°48′S 32°06′E	*C. papyrus*	S Afr.-1
83	South Africa	Table Mountain	29°35′S 30°30′E	*C. dives*	SE Afr.-1
300	South Africa	Mtunzini	28°57′S 31°39′E	*C. dives*	SA-4
301	South Africa	Mtunzini	28°57′S 31°39′E	*C. dives*	SE Afr.-1
302	South Africa	Cramond	29°24′S 30°25′E	*C. dives*	SE Afr.-1
718	Tanzania	TPC	03°31′S 37°20′E	Sugarcane	Tan-1
5	Uganda	KSW	01°35′N 31°36′E	?	WE Afr.-1
18	Uganda	KSW	01°35′N 31°36′E	Sugarcane	WE Afr.-1
8	Uganda	KSW	01°35′N 31°36′E	Sugarcane	WE Afr.-1
20	Uganda	KSW	01°35′N 31°36′E	Sugarcane	WE Afr.-1
215	Uganda	KSW	01°35′N 31°36′E	*C. papyrus*	Ugan-2
216	Uganda	KSW	01°35′N 31°36′E	*C. papyrus*	SE Afr.-2
217	Uganda	KSW	01°35′N 31°36′E	*C. papyrus*	Ugan-1
220	Uganda	KSW	01°35′N 31°36′E	Sugarcane	WE Afr.-1
221	Uganda	KSW	01°35′N 31°36′E	Sugarcane	SE Afr.-1
244	Uganda	KSW	00°16′S 32°31′E	Sorghum	WE Afr.-1
275	Uganda	KSW	01°35′N 31°36′E	?	WE Afr.-1
276	Uganda	KSW	01°35′N 31°36′E	?	WE Afr.-2
81	Zimbabwe	?	?	?	SE Afr.-2
82	Zimbabwe	?	?	?	Zimb-3

DNA No	Country	Location	Coordinate	Host Plant	Haplotype No
84	Zimbabwe	Chiredzi	21°0′S 31°38′E	Sugarcane	SE Afr.-1
229	Zimbabwe	?	?	?	S Afr.-1
230	Zimbabwe	?	?	?	Zimb-2
231	Zimbabwe	Chiredzi	21°0′S 31°38′E	Sugarcane	S Afr.-1
232	Zimbabwe	Chiredzi	21°0′S 31°38′E	Sugarcane	SE Afr.-1
235	Zimbabwe	?	?	?	S Afr.-1
236	Zimbabwe	?	?	?	SE Afr.-1
237	Zimbabwe	Chiredzi	21°0′S 31°38′E	Sugarcane	SE Afr.-1
238	Zimbabwe	Chiredzi	21°0′S 31°38′E	Sugarcane	Zimb-1
295	Zimbabwe	Hippo Valley Estate	21°0′S 31°38′E	Sugarcane	SE Afr.-1
296	Zimbabwe	Hippo Valley Estate	21°0′S 31°38′E	Sugarcane	SE Afr.-1
297	Zimbabwe	Hippo Valley Estate	21°0′S 31°38′E	C. digitatus	S Afr.-1
298	Zimbabwe	Hippo Valley Estate	21°0′S 31°38′E	C. digitatus	SE Afr.-1
299	Zimbabwe	Hippo Valley Estate	21°0′S 31°38′E	Typha latifolius	Zimb-1

?= No information, KSW = Kinyara Sugar Work, TPC= Tanzanian Planters Corporation

3.2.3.3 Molecular methods

Polymerase Chain Reaction (PCR) amplification was performed in a 50 µl volume containing 1X PCR buffer, 1.5mM MgCl$_2$, 200µM dNTPs, 15 pmol of each PCR primer, 1 unit of SuperTherm Gold *Taq* DNA polymerase (JMR Holdings, United Kingdom) and 1µl of genomic DNA. Primers used in the study were: Ron V (5'-GGAGCTCCAGATATAGCTTTCCC-3') and K525 (5'-ACTGTAAATATATGATGAGCTCA-3') (Loxdale and Lushai 1998) except for samples from Ghana, Nigeria, Tanzania, Ethiopia, Senegal and Mozambique (DNA No. 446-448 only), where the primer LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3')

(Folmer *et al.* 1994) was used instead of Ron V. PCR was performed using a Perkin Elmer GeneAmp PCR System 2400, under the following conditions: 94°C for 11 minutes (min), 30 cycles of (94°C for 30 seconds (s), 50-55°C for 30 s, 72°C for 30-90 s), 72°C for 7 min, 4°C hold. Amplified DNA was purified using the Qiagen QIAquick™ PCR purification kit following the manufacturer's protocol. DNA sequencing reactions were performed using the ABI PRISM® BigDye™ Terminator v3.0 Ready Reaction Cycle Sequencing Kit, cleaned using Ethanol/EDTA precipitation with slight modification of the manufacturer's protocol, and sequences were visualized on an ABI 3100 Genetic Analyzer.

3.2.3.4 Sequence analysis and phylogenetic reconstruction

Editing and assembling DNA sequence chromatograms was done using the Staden package (Staden 1996). Sequences were then aligned using ClustalX (Thompson *et al.* 1997) and manually corrected using BioEdit 5.0.9 sequence alignment editor (Hall 1999). A haplotype network was generated from the 502 bp alignment using the statistical parsimony method of Templeton *et al.* (1992) as implemented in TCS (Clement *et al.* 2000). Each haplotype was represented by a single sequence for phylogenetic analysis, which was performed by maximum parsimony (MP) and neighbour joining (NJ) in PAUP* v4.0b10 (Swofford 2002). The MP analyses used heuristic searches comprising 100 random addition sequences of taxa and tree-bisection-reconnection (TBR) branch swapping. MP bootstrap analysis employed 1000 replicates, each of which used 10 random addition sequences for taxa. Only bootstrap values greater than 50% are reported. MODELTEST version 3.7 (Posada and Crandall 1998) was used to select the substitution model that best describes the data. A NJ analysis was performed using maximum likelihood (ML) distances obtained using

parameter estimates derived from MODELTEST. Support for internal nodes was assessed by bootstrap analysis with 1000 replications.

3.2.4   Results

3.2.4.1 DNA sequence variation

Forty-five variable sites were found in the 502 bp fragment for which all specimens had data. Of these, 33 sites were parsimony-informative. Uncorrected pairwise sequence distances between haplotypes ranged from 0.20 to 4.98% (Table. 3.2). The percentages of Thymine (T), Cytosine (C), Adenine (A) and Guanine (G) was 39.0, 14.9, 31.1, and 15.0%, respectively.

Table 3.2   Percentage uncorrected pairwise distance in the COI gene within and among *Eldana saccharina* haplotype groups

	Southern African Group	West African Group	Rift Valley Group	South/East Group
Southern African Group	**0.20-1.00**			
West African Group	1.00-2.41	**0.20-1.59**		
Rift Valley Group	2.39-3.99	2.39-4.98	**0.40-3.59**	
South/East Group	1.59-2.79	2.19-2.79	2.39-4.58	**0.40-0.60**

3.2.4.2 Haplotypes

Twenty-three different haplotypes were detected from the 66 specimens included in the analysis of which 15 haplotypes were singletons (i.e. represented by single individuals). The haplotypes formed four distinct groups that are congruent with their geographic locations except for some of the specimens from eastern and southern Africa where geographically distant haplotypes grouped together (Figure 3.2). The most common haplotype (SE Afr.-1; Figure 3.2) was widespread in southern African countries and was represented in eighteen of the sequenced individuals from Mozambique, South Africa and Zimbabwe, and two individuals from East Africa (one from Kenya east of the Rift Valley and one from Kinyara Sugar Works in Uganda). This haplotype (SE Afr.-1; Figure 3.2) and another ten haplotypes (Zimb-1, Moz-1, Zimb-2, S Afr.-1, Moz-2, SA-1, SA-2, Moz-3, SA-3 and SA-4; Figure 3.2) that differ from each other by one to three mutational steps, were grouped together and referred to as the southern African Population (Table 3.2 and Figure 3.2). Two of the sequenced individuals from Zimbabwe and two individuals from Uganda were represented in three haplotypes forming a distinct group and referred to as South/East African Population (Table 3.2 and Figure 3.2). For individuals from West Africa, two distinct haplotypes (WE Afr.-1 and WE Afr.-2; Figure 3.2) that are different only by one mutational step were detected. Four haplotypes (Eth-1 to Eth-4) were detected in individuals from Ethiopia and they are somewhat closer to the West African haplotypes than to the southern or East African haplotypes. Rift Valley specimens from Kenya and Tanzania were represented by two haplotypes (Ken-1 and Tan-1; Figure 3.2) that are one mutational step from each other but which cannot be linked to other specimens at the 95% confidence level. One sequenced individual (Ugan-2; Figure 3.2) from Uganda could not be linked with other sequences in the study at 9 connection steps at 95% confidence levels.
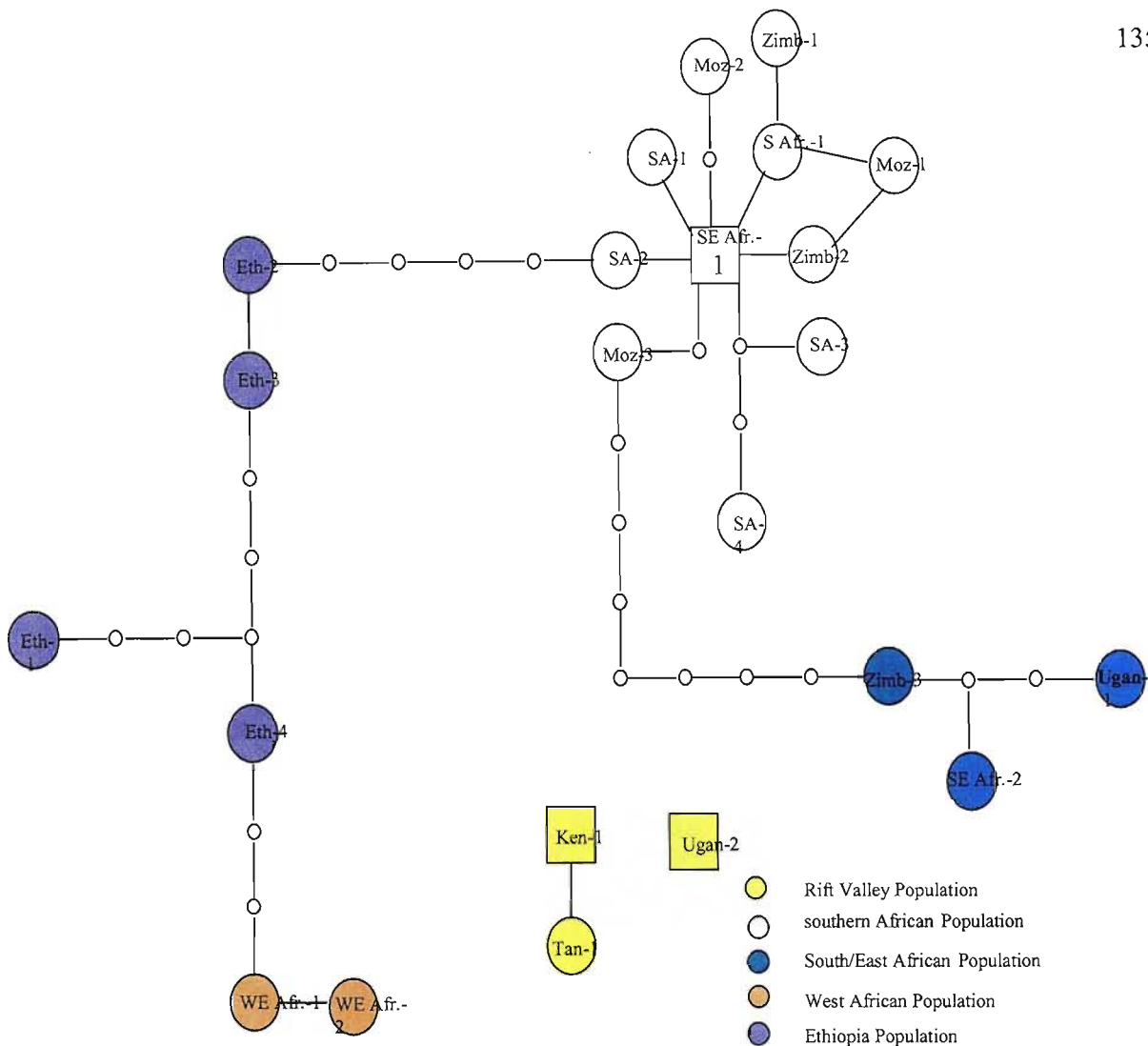
Figure 3.2 A TCS network showing the relationships between the 23 haplotypes. Geographic origins (Table 3.1 and Figure 3.1) and individuals (Table 3.1) are noted. Each small empty circle represents one hypothetical mutational step. Names before haplotype numbers are names of representative countries for that particular haplotypes. Haplotypes represented in samples from more than one country are given a representative region/s name. Zimb=Zimbabwe, SA=South Africa, Moz=Mozambique, Ken=Kenya, Eth=Ethiopia, Tan=Tanzania, Ugan=Uganda, S Afr.=Southern Africa, SE Africa= South/East Africa, WE Afr.= West East Africa.

3.2.4.3 Phylogenetic analysis

All the phylogenetic analyses performed supported four different groups of haplotypes that correspond to major biogeographic regions of Africa (Figure 3.2 and 3.3). The strict consensus of 12 MP trees (length = 64, consistency index = 0.7031, retention index = 0.8100) is shown in Figure 3.3a. Four groups are visible. The first group is a southern African group, also containing one individual from Uganda and one individual from eastern Kenya. This is the largest group comprising 47.8% of the haplotypes. The group shows within-group sequence divergence of up to 1.0% (Table 3.2). The second group includes all haplotypes from West Africa, (Benin, Senegal, Nigeria and Ghana) and Ethiopia with one sequenced individual from Uganda. This group further splits into three small groups, where the West African haplotypes with the individual from Uganda forms a group separated by branches with 78-82% bootstrap support. The Ethiopian specimens from Lake Tana form the second small group with weak bootstrap support of 67% and haplotypes from the Ethiopian Rift Valley form the third small group in this group. There is relatively high within-group sequence variation in this group (Table 3.2): 1.59% divergence between the Ethiopian Rift Valley haplotype 2 and haplotype WE Afr.-1 from Benin. The third group consists of one haplotype from Uganda plus Rift Valley haplotypes from Kenya and Tanzania. This group has the highest within-group variation (3.59%) and strong bootstrap support (79%; Figure 3.3). The fourth group has a mixed distribution of haplotypes from Uganda and Zimbabwe (Figure 3.3).
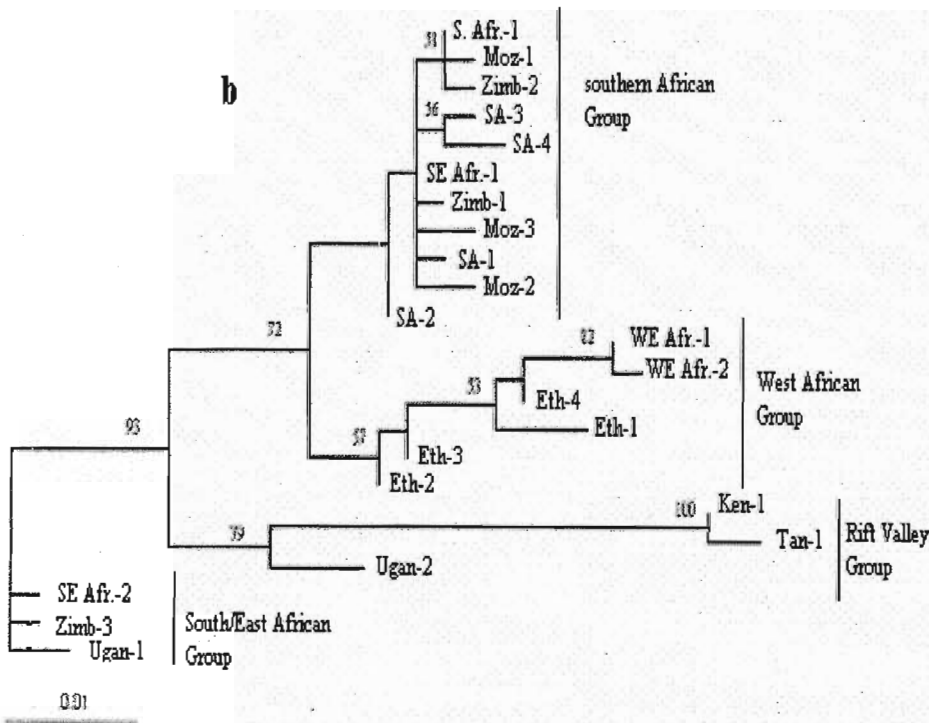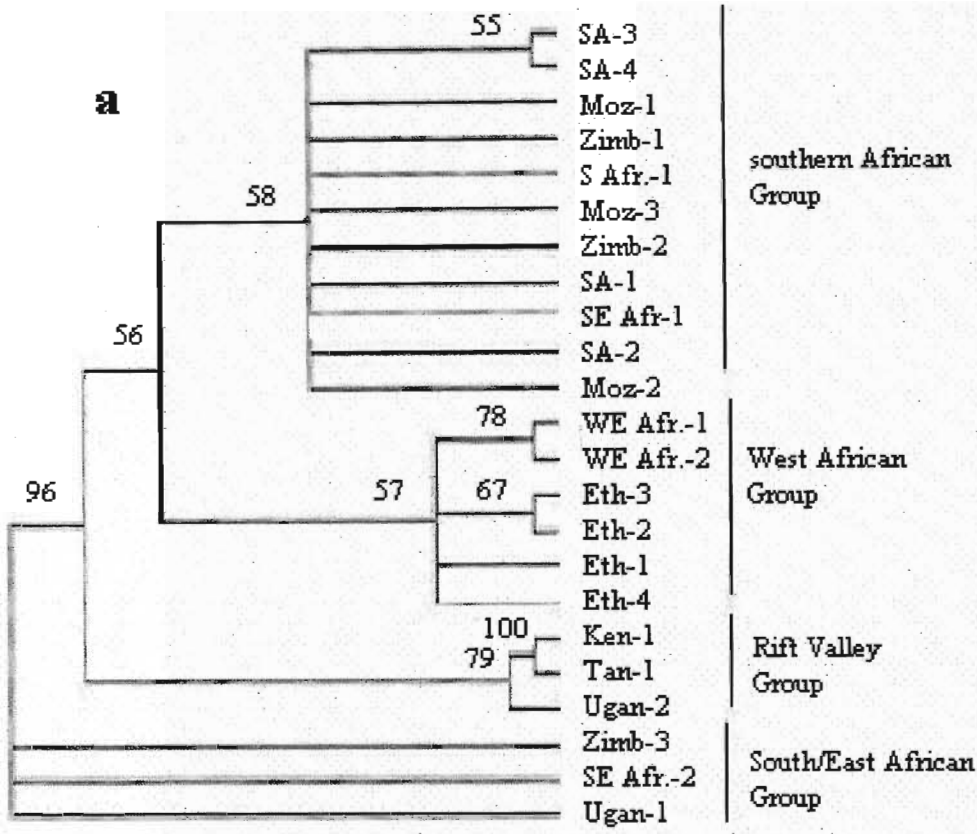
Figure 3.3. Unrooted phylogenetic trees showing the relationships among the 23 haplotypes; (a) strict consensus of 12 most parsimonious trees, (b) NJ tree. Numbers above internodes are MP/NJ bootstrap support values. Only bootstrap values greater than 50% are shown. Haplotype numbers are as indicated in Table 3. 1. Names before the haplotype numbers are names of representative counties for that particular haplotype. Haplotypes represented in samples from more than one country are given a representative region/s name. Zimb=Zimbabwe, SA=South Africa, Moz=Mozambique, Ken=Kenya, Eth=Ethiopia, Tan=Tanzania, Ugan=Uganda, S Afr.=southern Africa, SE Afr.=South/East Africa, WE Afr.=West/East Africa.

3.2.5 Discussion

3.2.5.1 Host plant associated genetic differentiation

Studies of generalist phytophagous insects often reveal that they represent complexes of genetically differentiated host races or cryptic species (Martel *et al.* 2003; Stireman *et al.* 2005). The African sugarcane borer, *E. saccharina*, feeds on a variety of host plants (Carnegie 1974; Betbeder-Matibet 1981; Conlong 1997; Polaszek and Kahn 1998; Conlong 2001; Mazodze and Conlong 2003). Wetland sedges (Cyperaceae, Juncaceae, Typhaceae) make up a large proportion of its natural host plants (Atkinson 1980; Conlong 2001; Mazodze and Conlong 2003) and it also occurs on a number of grasses (Poaceae) (Betbeder-Matibet 1981; Maes 1998). Ecological studies have reported that the insect feeds on different host plants in various parts of Africa (Conlong 2001; Matama-Kauma *et al.* 2002; Atachi *et al.* 2005). Molecular studies on the insect revealed the presence of genetic variation among populations of the pest from different parts of Africa (King *et al.* 2002; Assefa *et al.* 2006). The genetic differentiation between *E. saccharina* populations may be a result of specialization on different host species (Mitter *et al.* 1988, Farrell 1998) or isolation due to geographic barriers (Arctander *et al.* 1999; Alpers *et al.* 2004) or both.

Evidence in this study, however, argues against the existence of host plant associated races in *E. saccharina*. The first line of evidence for absence of host plant associated genetic differentiation is presence of the most common haplotypes on all host plants, in multiple samples, over vast geographic areas. The most common haplotype grouping (SE Afr.-1) was found widely distributed in the southern African countries and was represented in all host plant species included in the study (Table 3.1). The same is true for haplotypes WE

Afr.-1 and WE Afr.-2. These haplotypes were represented in maize, sorghum and sugarcane. The lack of a correlation between populations from different host plants and genetic distance indicates that *E. saccharina* attacking different hosts within geographically similar areas are not isolated from each other and the exchange of genetic material between *E. saccharina* populations attacking different plants is not blocked because of the expansion in host range. This effectively randomizes haplotypes in populations attacking different host plants. It is possible that haplotypes that occur on sedge hosts in the South may occur on grasses, which were not sampled. Also, ecological conditions in the West may have allowed *E. saccharina* populations to develop on a different host compared to the East and South. For example, *Cyperus papyrus* L. (Cyperaceae), which is the most common host of *E. saccharina* in the East and South is very scarce in areas west of Lake Chad (Beadle 1974a). Therefore, in the latter region, where the species is known from the abundant large grasses (Atachi *et al.* 2005), one is unlikely to find *E. saccharina* infesting *C. papyrus*.

The second line of evidence for absence of a host plant-associated lineage is the separation into different haplotype groups of individuals feeding on a particular host plant species. For example, *E. saccharina* individuals from *C. papyrus* are found in all haplotype groups. The same is true for *E. saccharina* from maize and sugarcane that are found in the southern African Group and West African Group. The conclusion that *E. saccharina* has not diversified into separate lineages on the different host plants in the same area implies that gene flow has been high enough to prevent the trade-offs in fitness between populations of *E. saccharina* attacking different host plants from creating isolation.

3.2.5.2 Impact of geography and climate on genetic differentiation

The largest portion of diversity revealed in this study is distributed in eastern Africa. High genetic diversity was observed between individuals from Kinyara Sugar Works Ltd. in Uganda and individuals from Kenya. The former individuals were distributed into all four haplotype groups detected in this study, as were the latter. The Kenyan specimens from the Rift Valley, east of the Rift Valley and west of the Rift Valley each fall into separate haplotype groups (Figures 3.2 and 3.3). Though the Ethiopian specimens were all in one haplotype group, there is an indication of genetic sub-structuring in the Ethiopian specimens. Ethiopian specimens from the Rift Valley were quite different from specimens collected from Lake Tana (Figures 3.2 and 3.3) located west of the Rift Valley (Figure 3.1). In contrast to the eastern African specimens, western and southern African specimens from different countries were genetically closer to each other. The relatively high genetic diversity in East African populations of *E. saccharina* as compared to populations from the rest of Africa could be associated with the impact of volcanic eruptions in the Miocene and Pleistocene that significantly altered the hydrology of the region (Beadle 1974b). Such massive volcanic eruptions and climatic change in the area could have modified the population structure of *E. saccharina*. Studies on vertebrate herbivores (Arctander *et al.* 1999; Alpers *et al.* 2004) and on *Busseola fusca* Fuller (Lepidoptera: Noctuidae) (Sezonlin *et al.* 2006) have provided evidence of the impact of these events on the distribution of different animal lineages in Africa.

3.2.5.2.1   Impact of the Rift Valley on E. saccharina *populations*

The relatively high level of population subdivision and significant pairwise genetic differentiation among *E. saccharina* populations in eastern Africa could indicate that the

species may have undergone population crashes in this region in the distant past (Arctander *et al.* 1999; Alpers *et al.* 2004; Sezonlin *et al.* 2006). Until the onset of rifting during the Miocene, the bulk of Africa was little affected by major earth movements like those which elsewhere pushed up the Alps and the Himalayas (Hamilton 1982a). In the Miocene, drastic earth movements affected the hydrology and biology of Africa (Beadle 1974b). The African Rift Valley that extends from lake Malawi, through the western and eastern rift of East Africa, into Ethiopia and on, further north, as far as the Red Sea and the Jordan Valley of western Asia, was the result of these geological events that started in the Miocene, but uplift continued in some areas until the terminal Pleistocene (Beadle 1974b). This rifting probably marks the initial stages of fragmentation of the African continent (Hamilton 1982a). The events in the Rift Valley greatly influenced the pattern of distribution of African herbivores (Arctander *et al.* 1999; Alpers *et al.* 2004) and insects (Sezonlin *et al.* 2006). This rift uplift could explain the distribution of genetic diversity in *E. saccharina* populations in East Africa. The separation between the Kenyan populations from the Rift Valley, east of the Rift Valley and west of the Rift Valley into three distinctly different groups strongly supports the hypothesis that the Rift Valley acted as a barrier to gene flow between *E. saccharina* populations in this area.

The climate of the eastern Rift Valley is relatively arid and most lakes in these Rift Valleys lack outlets (Hamilton 1982b). Severe aridity during the Quaternary drastically affected the eastern Rift Valley area. Lakes such as Nakuru in Kenya and the Afar and southern Ethiopian Rift Valley lakes were dry or nearly so (Coetzee and Bakker 1989). The lack of rainfall must have changed the vegetation pattern of the area. Plants in the region could only survive in small isolated water bodies. The *E. saccharina* populations in this eastern Rift Valley could therefore have been isolated from the rest of the populations in Africa

because of habitat fragmentation in the eastern Rift Valley. The genetic differentiation observed between the Ethiopian specimens from the Rift Valley and outside the Rift Valley may be explained by habitat fragmentation in that region, caused by climatic changes in the Pleistocene (Coetzee and Bakker 1989).

3.2.5.2.2 Uganda- Area of convergence or divergence of E. saccharina?

The large genetic diversity in Ugandan populations of *E. saccharina*, when compared to the southern and West African populations may be due to one or both of the following causes. The first cause could be volcanic eruptions that took place in the Miocene and Pleistocene that resulted in the formation of several small crater lakes in the western Rift Valley on the Congo-Uganda border. Some of these lakes had no outlet and were isolated from other water systems (Beadle 1974b). *Eldana saccharina* populations surviving in vegetation in these would thus have been isolated in and restricted to wetland plants around these shallow lakes. As a consequence of prolonged isolation, the extant populations of *E. saccharina* situated close to each other could be highly divergent.

However, the pattern of interactions between the lakes changed from time to time and some of these lakes became connected by rivers to other water systems (Beadle 1974b). It could be hypothesised that as wetland plants started to grow along the rivers, populations at the western limits of the refugial range would expand into the areas of suitable territory in West Africa and those at the southern limits would expand to southern Africa (Petit *et al.* 2003). The fact that Ugandan populations from *C. papyrus* are closely related to Zimbabwe populations (South/East African group) might be explained by the recent expansion of these populations to the south (Petit *et al.* 2003).

Another hypothesis suggests that *E. saccharina* populations may have been isolated from each other because of the volcanic eruptions and climatic changes in the Miocene and Pleistocene and survived in refugia in different parts of Africa. The populations of *E. saccharina* surviving in these refugia were subjected to different selection pressures, which allowed genetic divergence from each other because of prolonged isolation and lack of gene flow between them. However, with time, the discrete populations may have expanded and colonized suitable vegetation within their respective regions. They converged in Uganda because presently a network of swamps occupies about 6% of the total surface of Uganda (Wasawo 1964) and about sixty species of plants have been recorded in these swamps (Beadle 1974a). According to the resource concentration hypothesis, habitat diversification will result in an increase in the number of herbivores (Päts 1996). The diverse host plants available in a vast area of land are therefore likely to be invaded by the populations from west and south through the continuous link of water bodies in the western Rift Valley. Thus, the increased genetic diversity observed in the Ugandan *E. saccharina* population could be a result of dispersal of the western and southern population into Uganda. This country therefore could be a "hybrid zone" where the different populations of *E. saccharina* make contact (Hewitt 1999).

In contrast to eastern Africa where there have been numerous reversals in watercourses since the Miocene, the watercourses in the rest of the continent have never been disrupted (Beadle 1974b). Therefore it is likely that the lower genetic diversity observed within the southern African population (southern African group) and the West African population (West African group) is a result of habitat discontinuity and difference in climate between these regions, rather than past geological events.

3.2.5.3 Estimation of divergence time using a molecular clock

The mitochondrial DNA sequence divergence among the different *E. saccharina* populations suggests that the tectonics in the late Miocene (2.5 million years ago) and early Pleistocene (1.6 million years ago) played an important role in the current distribution of *E. saccharina* populations (Beadle 1974a). Divergence time estimations using a molecular clock of 2% substitution per million years suggest (Brown *et al.* 1979) the eastern rift population of *E. saccharina* (Rift Valley Group) diverged from the remaining groups in the late Miocene. The 4.98% sequence divergence observed between this group and the West African group indicates that these two groups diverged from each other around 2.5 million years ago. Populations in the eastern Rift Valley, therefore, were presumably separated as the Rift Valley started to come into being during the late Miocene. Similarly the mitochondrial DNA sequence variation between *E. saccharina* populations from the western Rift Valley (South/East African group) and the other groups (southern African and West African Groups) was as high as 2.79%, which coincides with the geological events in the area. Volcanic eruptions in the early Pleistocene resulted in the formation of hundreds of isolated lakes in the western Rift Valley that gradually interconnected in the late Pleistocene (Beadle 1976a). These volcanic eruptions, therefore, could be the reasons for separation of the South/East African population of *E. saccharina* from the southern African and West African populations.

3.2.6 Conclusions

Results of the current study show the existence of considerable genetic diversity among populations of *E. saccharina* from different parts of Africa. It is proposed that extreme geographic perturbations that occurred in East Africa during the Miocene and then habitat linkages re-establishing during the late Pleistocene were the main drivers of this genetic diversity. The eastern Rift Valley seems to act as a geographic barrier against gene flow between *E. saccharina* populations. Specimens representing all four populations were found in Uganda. This region was suggested to be a hot spot and/or a melting pot for *E. saccharina* populations in Africa. No host plant associated genetic differentiation was observed. It is suggested that sampling populations of *E. saccharina* from geologically older wetlands such as the Sudd in Sudan, the Okavango Swamps in Botswana, and Lake Chad in Chad may provide more solid evidence on the relative ages of the *E. saccharina* populations, which would either substantiate or repudiate the current assumptions on diversification of *E. saccharina* populations.

3.2.7 References

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3.3 *BUSSEOLA* SPECIES COMPLEX (LEPIDOPTERA: NOCTUIDAE) IN ETHIOPIAN SUGARCANE: IDENTIFICATION BASED ON MITOCHONDRIAL DNA DATA

3.3.1 Abstract

In surveys conducted in sugarcane in various parts of Ethiopia in 2003/4, *Busseola* species were the predominant stem borers of sugarcane in most of the fields evaluated. Sequence divergence in the mitochondrial cytochrome-*c* oxidase I (COI) gene was used as a tool to identify and separate the species of *Busseola* attacking Ethiopian sugarcane. Partial sequences from the COI region of the mitochondrial DNA of Ethiopian specimens were compared with sequences of already identified noctuid species from the East African region. Results of the sequence analysis indicated that the *Busseola* species in Ethiopian sugarcane belonged to *B. fusca* and *B. phaia*. Sequence divergences between Ethiopian *Busseola* species was as high as 5.02%, whereas divergences within species were generally less than 1% in both species identified. This DNA based method of identification proved to be a powerful tool for identifying species of *Busseola*. It was reliable in the present case because previous intensive sampling of insects in several countries of East Africa resulted in good knowledge of the intraspecific molecular variability of the species. The regular use of the method, together with the more traditional morphological taxonomic approach, is strongly recommended.

3.3.2 Introduction

Noctuidae is one of the largest lepidopteran families, encompassing about 20 000 species (Holloway 1998). The number of described species of this family known to be cereal stem borers in the Afro-tropical region amounts to 157 (Moyal 2006) of which the most economically important species belong to the genera *Busseola* and *Sesamia*. Accurate identification of pest species is the first and most fundamental step to developing sound pest management strategies (Szalanski *et al.* 2003). However, many of these stem borers are closely related and are difficult to distinguish from each other morphologically, and no key is available to cover all noctuid stem borers (Holloway 1998), nor their different life stages.

Misidentifications of these pests have occurred frequently, resulting in the publication of false data, which are perpetuated often for decades (Polaszek 1992). This problem is aggravated when identification of larvae is considered. Morphological structures such as setae are easily broken and frequently are missing from alcohol-preserved and deep-frozen materials. This makes identification difficult and results unreliable (Meijerman and Ulenberg 1998). Moreover, even when all setae are present, differences between species at the larval stage are small and often it is not possible to identify larvae. For instance, in the case of noctuid stem borers, species belonging to different genera, *e.g. Busseola fusca* Fuller (Lepidoptera: Noctuidae) and *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae), can have exactly the same long and microscopic setae, and the only difference in larval morphology is a slight change in the position of two setae on one segment (Moyal and Tran 1989). Distinction of larvae of species within a genus is generally not possible.

The use of DNA-based technologies has been suggested as the best option to solve the current problems in identification of field-collected materials (Hogg and Hebert 2004). Although this approach is not without controversy (Cognato 2004), the lack of adequate morphological taxonomic services makes the molecular approach an attractive alternative. Hebert *et al.* (2003a) proposed that the analysis of sequence diversity in the cytochrome-*c* oxidase I (COI) gene of mitochondrial DNA can serve as the core of a global identification system in the animal kingdom. Specifically they suggest the employment of DNA sequences as taxon "barcodes" and propose the COI gene to serve as a base for identification of all animal life. The potential success of barcoding was illustrated by an analysis showing deep genetic divergences present in 13 000 closely allied species from a range of animal phyla (Hebert *et al.* 2003b). Analysis of COI profiles of single individuals from 200 lepidopteran species verified that the system is 100% successful in correctly identifying subsequent specimens of these species (Hebert *et al.* 2003a). However, these papers did not adequately address the important question of whether the COI gene has the discriminatory power to correctly identify closely related species.

This study is focused on the identification of Ethiopian *Busseola* species utilizing COI barcoding. This system was used for the identification of field-collected larvae that died before reaching adulthood. Identification of species using larval morphology was not possible as reliable keys were not available. Previously identified adult specimens of *Busseola* species (Moyal unpublished) from East Africa were used as a reference for the identification of the Ethiopian *Busseola* species from sugarcane.

3.3.3 Materials and Methods

3.3.3.1 Insect specimens

Material examined (Table 3.3) was collected from sugarcane and indigenous host plants bordering sugarcane fields of peasant farmers and commercial estates in Ethiopia between November 2003 and February 2004. The immatures were kept in sugarcane stalks and/or artificial diet to complete their development until adulthood, as detailed in Chapter 2.3. Unfortunately none of the collected specimens developed to the adult stage. The dead larvae were taken out of the stalks and/or artificial diet and kept frozen in 95% alcohol in sealed 30ml glass vials until representative specimens could be used for DNA-based identification.

3.3.3.2 DNA extraction

Genomic DNA was extracted from individual larval thoraces using the Qiagen DNeasy™ Tissue Kit as recommended for animal tissues and the extracted DNA was stored at -20°C until required for amplification. Voucher specimens are housed at the South African Sugarcane Research Institute (SASRI), Mount Edgecombe, KwaZulu-Natal, South Africa.

3.3.3.3 DNA amplification and sequencing

Polymerase Chain Reaction (PCR) amplifications were performed in a 50 µl volume containing 5µl of 10X PCR buffer, 1µl of dNTP mix (10 µM of each dNTP), 15 pmol of each PCR primer, 1U of SuperTherm Gold *Taq* DNA polymerase and approximately 1µl of genomic DNA. PCR was performed using a Perkin Elmer GeneAmp PCR System 2400

using the following conditions: denaturation at 95°C for 11 minutes (min) followed by 35 cycles of (94°C for 30 seconds (s), 50°C for 30 s, 72°C for 90 s) and a final hold at 4°C. The PCR primers used were LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3')(Folmer *et al.* 1994). Successful amplification was confirmed by examining a 5µl aliquot of the amplification product using agarose gel electrophoresis. Amplified DNA from individual specimens was purified using the Qiagen QIAquick™ PCR purification kit, following the manufacturer's protocol. Cleaned PCR products were then cycle-sequenced using the ABI PRISM® BigDye™ Terminator v3.0 Ready Reaction Cycle Sequencing Kit following the manufacturer's recommended conditions. The sequencing reactions were cleaned using Ethanol/EDTA precipitation, with minor modification on the manufacturer's protocol, and visualized on an ABI 3100 Genetic Analyzer.

Table 3.3   Localities in Ethiopia from where specimens of *Busseola* species were collected.

DNA No	GenBank Acc. No.	Farm Type	Location	Position	Host plant
415	DQ337201	Estate	Wonji	39°12′E; 08°31′N	*Cyperus dives*
450	DQ337199	Peasant	Inguti	37°06′E; 11°24′N	Sugarcane
451	DQ337196	Peasant	Goma	36°36′E;07°51′N	Sugarcane
452	DQ337195	Peasant	Sidama	38°26′E; 06°54′N	Sugarcane
715	DQ337200	Peasant	Mankusa	37°11′E; 10°40′N	Sugarcane
716	DQ337198	Peasant	Mankusa	37°11′E; 10°40′N	*Pennisetum purpureum*
717	DQ337197	Peasant	Goma	36°36′E;07°51′N	Sugarcane

3.3.3.4 Sequence analysis

Editing and assembling DNA sequence chromatograms was completed using the Staden package (Staden 1996). Sequences were then aligned using ClustalX (Thompson *et al.* 1997) and manually corrected using BioEdit sequence alignment editor (Hall 1999). The sequences of the Ethiopian specimens were first compared. A phylogenetic analysis of the samples was performed by Maximum Parsimony (MP) using an exhaustive search. Node support was assessed by the bootstrap method with 1000 replications of exhaustive searching using PAUP* v4.0b10 (Swofford 2002). *Sesamia nonagrioides* Lef. (Lepidoptera: Noctuidae) sequences were downloaded from GenBank (GenBank accession number AY649322 and AJ829718) and used as an out-group in the analysis. These DNA sequences were then sent to the Institut de Recherché pour le Développement (IRD) in France to be linked with sequences of morphologically identified specimens.

The last 343 bp of the COI fragment were compared with the sequences obtained from insects collected for a phylogenetic study of African noctuid stem borers that is presently underway (Moyal unpublished).

3.3.4 Results

3.3.4.1 Identification of Busseola *specimens*

3.3.4.1.1 The Ethiopian specimens

A phylogenetic analysis of the 658 base pair (bp) COI sequences produced for the Ethiopian *Busseola* specimens separated the specimens into two groups. Group one contained four sequences (716, 450, 715 and 415) differing from one another by 2-5 bp's (i.e., 0.32-0.77% divergence) (Figure 3.4 and Table 3.4). Group two contained three sequences (452, 717 and

451) differing from one another by 0-1 bp's (i.e., 0-0.2% divergence) (Figure 3.4 and Table 3.4). Groups one and two differed from one another by an average of 33 bp's (5.02% divergence). This result suggested that two species of *Busseola* were present in our sample. These DNA sequences were sent to IRD in France to see if they could be matched with data for overlapping COI sequences of *Busseola* species.
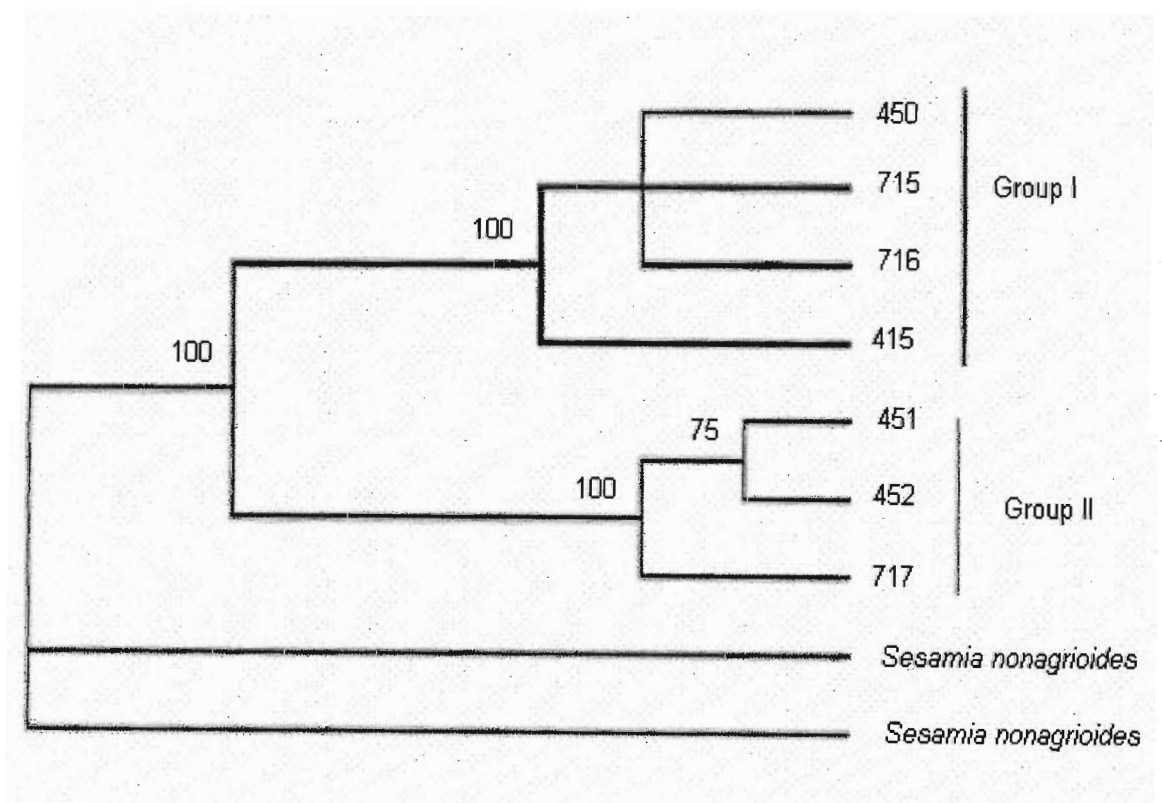
Figure 3.4   Cladogram representing relationships in *Busseola* specimens from sugarcane fields of Ethiopia. Numbers above internodes are bootstrap support values. Numbers in terminal branches are DNA numbers indicated in Table 3.3.

Table 3.4   Percentage uncorrected pairwise distance observed in COI gene within and between *Busseola* specimens from Ethiopia

	Busseola Group I	*Busseola* Group II
Busseola Group I	**0.32-0.77**	
Busseola Group II	4.56-5.02	**0.00-0.15**

3.3.4.1.2   *Comparison of the sequences with those from morphologically identified species*

The sequences of the two groups (last 343 bp of the COI fragment) matched exactly the sequences obtained from two species collected in East Africa: *B. fusca* and *B. phaia* (Figure 3.5). Three sequences (452, 717 and 451) belonged to the same haplotype as an identified *B. phaia* from Uganda (p-distance=0), and specimen 715 from Ethiopia belonged to the same haplotype as an identified *B. fusca* from Kenya, whereas the other specimen (450) differed by 0.3% (1 difference in 343 nucleotides). Sequences 415 and 716 have also found to be *Busseola fusca*.
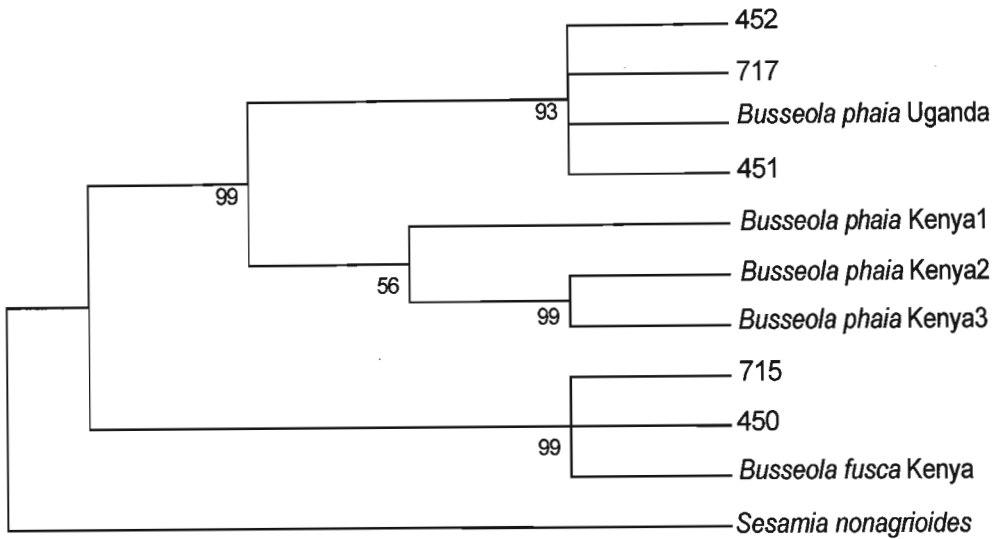
Figure 3.5   Cladogram including specimens from Ethiopia and identified species of *Busseola* from East Africa. Numbers below internodes are bootstrap support values.

3.3.4.2   Distribution of Busseola *species complex in sugarcane fields of Ethiopia*

After identification of the specimens, collection localities were mapped using ArcView program to determine the distribution of the two species in the country. Specimens collected from the northern and central part of the country were all *B. fusca* whereas specimens from the southern and western part of Ethiopia all were *B. phaia* (Figure 3.6).
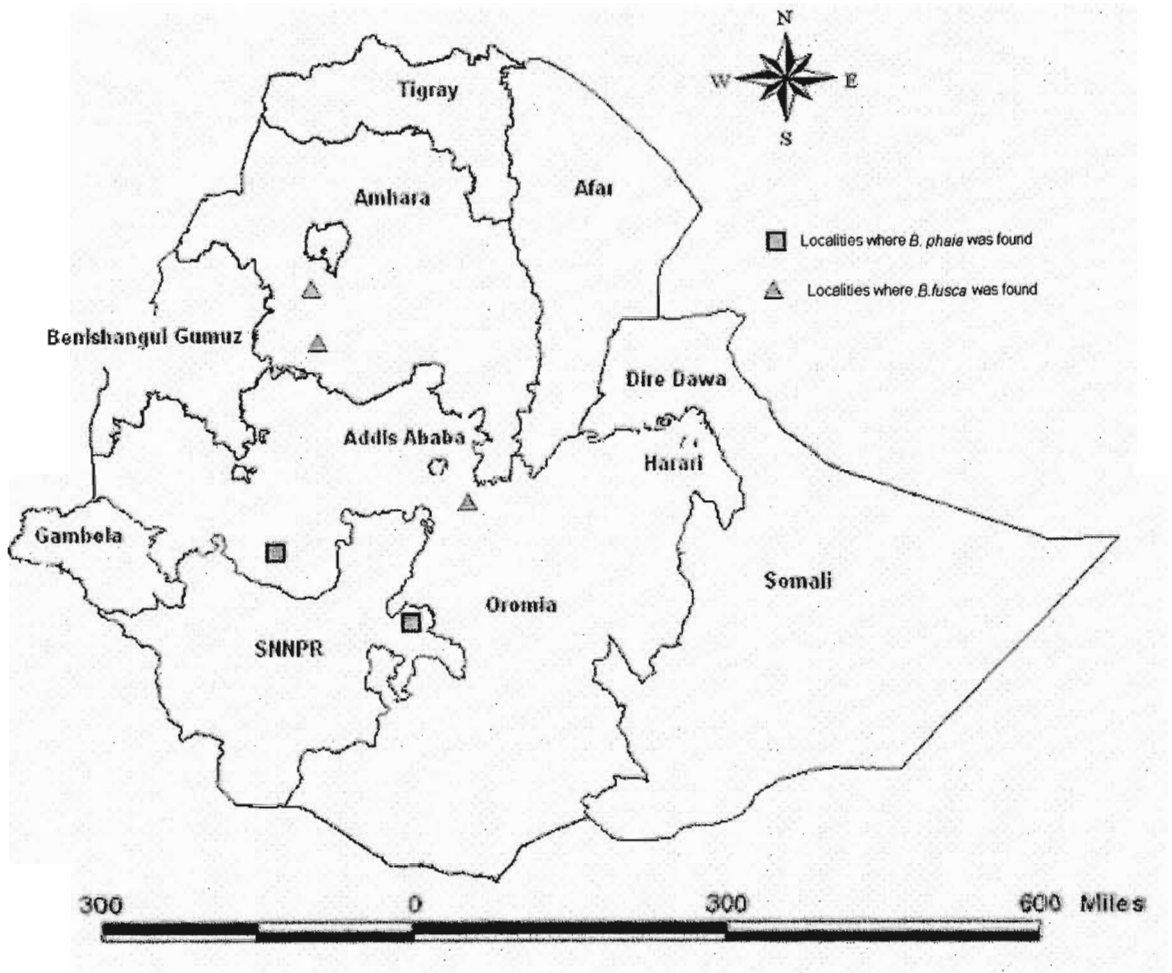
Figure 3.6 Map of Ethiopia showing localities where sugarcane and indigenous host plants were found infested by *Busseola* species. Regions of Ethiopia are named in bold. The indigenous host plants and locality names are listed in Table 3.3.

3.3.5 Discussion

3.3.5.1 Identification of Busseola *specimens*

DNA sequence analysis of the COI fragment of mitochondrial DNA was successful in discriminating the two species of *Busseola* in Ethiopia. Levels of divergence within species

were less than 1%, whereas inter-species divergence exceeded 4.5%. Similar levels of divergence have been reported in other studies of lepidopterans (Cognato 2004).

DNA-based methods in this case were found to be a quick, easy and reliable method for identification of species. This method may be a solution for conditions in Africa where there is an acute shortage of experts and rearing facilities to keep field collected insects alive until adult emergence for morphological identification. The use of barcoding as a taxonomic tool has however been criticized by many authors (e.g. Lipscomb *et al.* 2003; Will and Rubinoff 2004; Lee 2004), and it can result in misidentifications, particularly in closely related species. It must be stressed that the method was successful here because an exhaustive study of the ecology, morphology and molecular systematics of African noctuid stem borers is presently underway (Moyal unpublished) which has provided sufficient data for comparison of intraspecific and interspecific variability, and in which adults of many of the species could be correctly identified morphologically from carefully curated adult specimens, from which DNA samples were also obtained and sequenced..

First results obtained for a genus close to *Busseola, Manga* Bowden, showed the complexity of the evolutionary history of these insects in the past five million years (Moyal and Le Rü 2006). Several fragmentation events occurred as a result of paleo-climatic events, host-plant specialization, and geographic barriers such as the Rift Valley. This resulted in the formation of several distinct clades within species and explains the complexity of the systematics of this group. In the case of *B. fusca*, the first results (Sezonlin *et al.* 2006) showed only a recent main fragmentation event, resulting in three clades, one from West Africa and two from East Africa, showing about 2% divergence in the mitochondrial gene Cytochrome b. The situation is different with *B. phaia*, whose

evolutionary history is more complex and similar to what was observed in *Manga* species. The pairwise distance between the two species *B. fusca* and *B. phaia* for the overlapping 343 bp region of COI is between 5.5% and 7.4%. But within *B. phaia*, the distance between the Ethiopia-Uganda group and the other clades is 3.3- 3.9%, and the distance between the Kenyan clades is 4.6-4.9%. If the sampling of *B. phaia* had been limited to the Kenyan specimens, it would have been difficult to ascertain that the Ethiopian insects belonged to this species because the genetic distance is high, indicating an ancient fragmentation event (Moyal unpublished). Because intensive sampling was done in different countries, Moyal (unpublished) found the same haplotype in Uganda as in Ethiopia, and this, combined with the knowledge on the ecology and systematics of the genus ascertained that the larvae from Ethiopia belong to the same species.

Further studies are necessary to decide if *B. phaia* is in fact a complex of different species. This species appears to be rather variable in morphology, which initially lead Bowden (1956) to describe two species, *B. phaia* and *Busseola segeta* Bowden, the first one located in the southern part of East Africa (Southern Zimbabwe) whereas the latter was found in Uganda. However, Nye (1960) found there was a morphological cline between these regions and stated that there was only one species, *B. phaia*, which includes two subspecies, *B. phaia phaia* and *B. phaia segeta*. The presence of this cline makes it difficult to identify the subspecies. The morphological and molecular study presently underway should help in clarifying this question (Moyal unpublished). It may be that the examination of many specimens will identify reliable morphological differences, but it is also possible that speciation has not occurred, or has just occurred without the accumulation of visible morphological differences.

3.3.5.2 Distribution of Busseola species complex in sugarcane fields of Ethiopia

Results of the sequence analysis in this study revealed that the *Busseola* species in sugarcane from the northern and central part of the country belongs to *B. fusca* whereas sugarcane in the south and western part of Ethiopia harbours *B. phaia*. *Busseola fusca* has been recorded from sugarcane fields in other parts of Africa, but never at pest levels (Polaszek and Khan 1998; Conlong 2000) and *B. phaia* has never been recorded as a pest in crop fields (Nye 1960). In the results of an extensive survey reported by Nye (1960), the *B. phaia* species complex was common in indigenous host plants and was rarely found in maize fields adjacent to infested indigenous host plants. Recent surveys in Kenya, however, showed that *B. phaia* is becoming common in maize fields, showing the potential of this insect to turn into a serious pest (Le Rü personal communication). It is assumed that indigenous hosts adjacent to cultivated crops can provide important refuges for both borers and their natural enemies (Polaszek and Khan 1998). However, information on the diversity and abundance of stem borers and their natural enemies in natural habitats in Ethiopia is absent. Further studies on host plant-stem borer-natural enemy associations under different local conditions are needed for a better understanding of the role of wild habitats as a source of pests and natural enemies on adjacent crops.

3.3.6   Conclusions

The molecular data revealed the presence of two species of *Busseola* in Ethiopian sugarcane. This is the first time that *B. phaia* is reported from sugarcane. The occurrence of this insect in Ethiopia was not previously reported. It is important to conduct regular surveys in sugarcane growing regions of the country to have a better understanding of the ecology of the insect and to facilitate in its management. The DNA based method of

identification proved to be a useful tool for identifying species of *Busseola*. The regular use of the method, together with the more traditional morphological taxonomic approach, is important for a better understanding of the diversity of stem borers in sugarcane.

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3.4 THE ESTABLISHMENT OF *COTESIA FLAVIPES* CAMERON (HYMENOPTERA: BRACONIDAE) IN SUGARCANE FIELDS OF ETHIOPIA AND THE ORIGIN OF THE FOUNDING POPULATION

3.4.1 Abstract

Cotesia flavipes Cameron has been introduced into several African countries for the control of *Chilo partellus* Swinhoe in maize and sorghum. It has never been released in Ethiopia, but is commonly found in maize and sorghum fields of the country. It is hypothesized that *C. flavipes* spread into Ethiopia from releases against *C. partellus* in Kenya and Somalia. This paper reports the recovery of *C. flavipes* from *C. partellus* in Ethiopian sugarcane more than 2000 km from the nearest known release sites in Kenya and Somalia. DNA sequences of the mitochondrial gene cytochrome oxidase subunit I (COI) were conducted on the Ethiopian specimens from sugarcane and specimens of *C. flavipes* from different countries of Africa that originated from a Kenyan laboratory colony to determine the origin of the Ethiopian population. In addition, partial DNA sequences of the COI gene were compared among African specimens of *C. flavipes* and those from other continents. The *C. flavipes* population established in Ethiopian sugarcane is closely related to the populations released against *C. partellus* in maize in other parts of Africa, which are related to the original Pakistani founder population. Dispersal of the parasitoid was estimated to be more than 200 km per year.

3.4.2 Introduction

Cotesia flavipes Cameron (Hymenoptera: Braconidae) is a gregarious endoparasitoid of lepidopteran stem borers of gramineous plants (Overholt *et al.* 1994). It is indigenous to South-East Asia (Mohyuddin 1971) from where it was imported to the New World for use against *Diatraea saccharalis* Fabricius (Lepidoptera: Pyralidae) in sugarcane (Gifford and Mann, 1967; Fuchs *et al.*, 1979), and redistributed in the Old World for the use against *Chilo* spp. (Greathead, 1971; Mendonca *et al.*, 1977; Mohyuddin *et al.*, 1981; Overholt, 1998) in maize and sorghum. This braconid larval parasitoid was released against *C. partellus* in Kenya, Uganda and Tanzania for 5 years between 1968 and 1972 by the International Institute of Biological Control (IIBC) but failed to establish (Overholt *et al.*, 1994; Overholt, 1998). *Cotesia flavipes* was re-introduced from Pakistan to Kenya against *C. partellus* in 1993 (Overholt *et al.*, 1994) and has become permanently established on this borer in maize fields of the country (Omwega *et al.*, 1997; Songa *et al.*, 2001). An impact assessment study conducted in Kenya by Zhou *et al.* (2001) demonstrated that the parasitoid had caused a significant reduction in the density of the exotic stem borer *C. partellus*. Following the success in Kenya, *C. flavipes* from the same laboratory colony in Kenya was released in Mozambique in 1996, and Uganda and Somalia in 1997 (Overholt, 1998) and results showed that the parasitoid had colonized maize fields. Releases of the parasitoid were then made in many countries in East and southern Africa and successful establishment of the parasitoid was reported from Mozambique (Cugala and Omwega 2001; Cugala *et al.* 2001), Tanzania (Omwega *et al.* 1997) and Uganda (Matama-Kauma *et al.* 2001). Though not released in Ethiopia, the parasitoid was reported to have established on three major stem borers, *Busseola fusca* Fuller (Lepidoptera: Noctuidae), *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) and *C. partellus,* in maize and sorghum

fields of the country (Getu *et al.* 2001) and it was consequently concluded that the parasitoid spread into Ethiopia from a founding population that had been intentionally released in Somalia and Kenya to control *C. partellus* in maize (Getu *et al.* 2003). In surveys carried out in 2003 and 2004 in sugarcane producing regions of Ethiopia, *C. flavipes* was recovered from *S. calamistis* in the Central region and up to 50% parasitism was recorded on *C. partellus* in sugarcane in the northern parts of the country (See Chapter 2.4).

The presence of *C. flavipes* in sugarcane fields of Ethiopia, more than 2000 km from the closest known release sites in Kenya and Somalia (Overholt, 1998), evoked a question on the origin of the founding population. The dispersal rate of this parasitoid was estimated to be 60 km per year (Omwega *et al.*, 1997). Using this model, the parasitoid would need at least 25 years to reach this area from the original release sites, or spread more than three fold faster than hypothesized. Moreover, the parasitoid is reported to have developed ecological strains that are adapted to searching specific host plants infested by stem borers (Mohyuddin *et al.*, 1981; Mohyuddin, 1991; Smith *et al.*, 1993). *Cotesia flavipes* collected from *C. suppressalis* Walker (Lepidoptera: Pyralidae) in rice in Japan was imported into Pakistan and reared on *C. partellus* feeding on maize. This rice/maize strain of *C. flavipes* successfully colonized *C. partellus* in sorghum and maize but did not attack *C. partellus* that infested sugarcane. However, sugarcane adapted strains of *C. flavipes* from Barbados, Indonesia and Thailand were subsequently established on *C. partellus* in sugarcane in India (Mohyuddin 1991). Similar results were obtained in the study conducted by Mohyuddin *et al.* (1981) where a Pakistani strain of *C. flavipes* maintained on *C. partellus* larvae in maize was readily attracted to maize stems but not to sugarcane. Based on results from their studies, these authors emphasized the need to give consideration to parasitoid plant

preference as well as host preference and suitability during attempts to introduce *C. flavipes*. The maize/sorghum strain of *C. flavipes* released in various countries of Africa showed pronounced success in colonizing maize and sorghum fields (Omwega *et al.* 1997; Cugala and Omwega 2001; Cugala *et al.* 2001 Matama-Kauma *et al.* 2001). However, its establishment in sugarcane fields has not been reported. It was thus thought the *C. flavipes* that established in sugarcane fields of Ethiopia is a sugarcane strain that might have been accidentally introduced from elsewhere. To test this hypothesis, molecular analyses were conducted using specimens of *C. flavipes* from sugarcane in Ethiopia and *C. flavipes* specimens from different parts of Africa that were released from the Kenyan colony. These African populations of *C. flavipes* were then compared with populations of the parasitoid from different hosts/habitats from different parts of the world. This study reports the results of the molecular analysis and the sources of the founding population of *C. flavipes* in Ethiopian sugarcane.

3.4.3 Materials and Methods

3.4.3.1 Insect specimens

Ethiopian *C. flavipes* specimens used in this study were from samples recovered from *C. partellus* in sugarcane at Girana (39°43′E; 11°34′N) in December 2004. The remaining African *C. flavipes* specimens were collected from *C. partellus* in maize. *Cotesia flavipes* was reared by the International Centre for Insect Physiology and Ecology (ICIPE), Kenya from where they were distributed for release into several African countries. Field collected specimens from five African countries (Kenya, Somalia, Tanzania, Zambia and Mozambique) were returned to ICIPE for identification. Specimens of *C. flavipes* from these countries were compared with the Ethiopian specimens. The origin and host/habitat

information of *C. flavipes* specimens out of Africa is provided in Table 3.5 along with the GenBank accession numbers for the DNA sequence data. The *Cotesia glomerata* L. specimen was from the University of Adelaide Insect Collection, Australia.
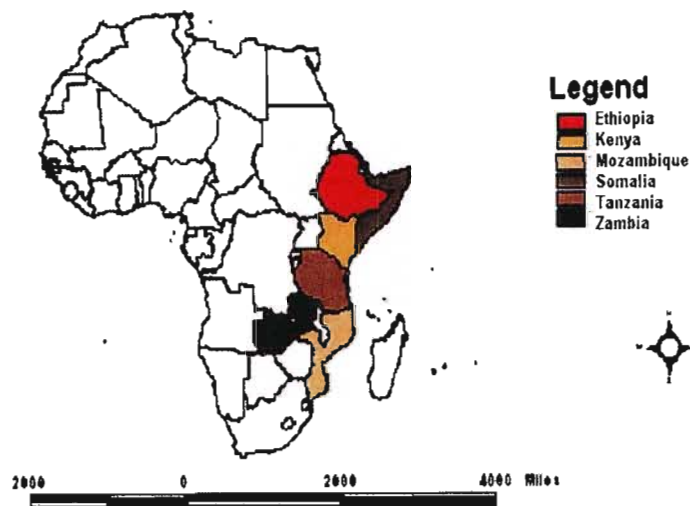
Figure 3.7   Map of Africa showing countries from where African *Cotesia flavipes* populations were collected.

3.4.3.2 DNA extraction

Genomic DNA was extracted from individual whole wasps using the Qiagen DNeasy[TM] Tissue Kit as recommended for DNA isolation from animal tissue. Genomic DNA was eluted in 50 µl of 10 mM Tris buffer and stored at -20°C until required for amplification. Voucher specimens are kept at the South African Sugarcane Research Institute (SASRI), Mount Edgecombe, South Africa.

3.4.3.3 DNA amplification and sequencing

Fragments of the cytochrome oxidase subunit I (COI) gene were amplified by the Polymerase Chain Reaction (PCR) using PCR primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al.* 1994). PCR amplification was performed in a 50 µl volume containing 1X PCR buffer, 1.5 mM MgCl$_2$, 200 µM dNTPs, 15 pmol of each PCR primer, 1 unit of SuperTherm Gold *Taq* DNA polymerase (JMR Holdings, United Kingdom) and 1 µl of genomic DNA, using a Perkin Elmer GeneAmp PCR System 2400. The reaction conditions used for most of the samples were: 94°C for 11 minutes (min), 30 cycles of (94°C for 30 seconds (s), 50-55°C for 30 s, 72°C for 30-90 s), 72°C for 7 min, 4°C hold. In some samples these reaction conditions failed to produce a result. For those cases, the following conditions were used: denaturation at 95°C for 11 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s and extension at 72°C for 90 s and hold at 4°C. Successful amplification was confirmed by examining a 5µl aliquot of the amplification product using agarose gel electrophoresis. Amplified DNA from individual specimens was purified using the Qiagen QIAquick™ PCR purification kit, following the manufacturer's protocol. Samples were then cycle sequenced using an ABI PRISM® BigDye™ Terminator v3.0 Ready Reaction Cycle Sequencing Kit following the manufacturer's recommended conditions. Completed reactions were cleaned using ethanol/EDTA precipitation with slight modification of the manufacturer's protocol, and sequences were visualized on an ABI 3100 Genetic Analyzer.

3.4.3.4 Sequence analysis

Editing and assembling DNA sequence chromatograms was completed using the Staden package (Staden 1996). Sequences were then automatically aligned using ClustalX (Thompson *et al.* 1997) and manually corrected using BioEdit (Hall 1999). A haplotype network was reconstructed using the statistical parsimony method of Templeton *et al.* (1992) in TCS 1.21 (Clement *et al.* 2000). Each haplotype was represented by single sequence for phylogenetic analysis, which was performed by Maximum Parsimony (MP) in PAUP* v4.0b10 (Swofford 2002). The MP analyses used a heuristic search, with tree reliability assessed by bootstrap analysis with 1000 replications. Only bootstrap values greater than 70% are reported. *C. glomerata* was used as the outgroup because it belongs to the same genus as *C. flavipes*.

Table 3.5   Collection locations, host insect, host plant and GenBank accession numbers for *C. flavipes* specimens used in this study.

Locality	Host insect	Host plant of insect host	Acc. number	Haplotype	Source
Thailand	*Chilo tumidicostalis* Hampson (Lepidoptera: Crambidae)	Sugarcane	DQ232340	HT_3	K. Muirhead (Univ. Adelaide, Aus.)
Piracicaba, Brazil	*Diatraea saccharalis* (Lepidoptera: Crambidae)	Sugarcane	DQ232320	HT_1	K. Muirhead (Univ. Adelaide, Aus.)
India	*Chilo partellus* Swinhoe (Lepidoptera: Crambidae)	Maize	DQ232336	HT_1	K. Muirhead (Univ. Adelaide, Aus.)
Florida, USA	*Diatraea saccharalis*	Sugarcane	DQ232330	HT_4	K. Muirhead (Univ. Adelaide, Aus.)

Mandeville, Jamaica	*Diatraea saccharalis*	Sugarcane	DQ232340	HT_2	K. Muirhead (Univ. Adelaide, Aus.)
South Pakistan	*Chilo partellus*	Maize	DQ232335	HT_2	K. Muirhead (Univ. Adelaide, Aus.)
Sri Lanka	*Chilo sacchariphagus* Bojer (Lepidoptera: Crambidae)	Sugarcane	DQ232327	HT_5	K. Muirhead (Univ. Adelaide, Aus.)
Labour-donnais, Mauritius	*Chilo sacchariphagus*	Sugarcane	DQ232319	HT_6	K. Muirhead (Univ. Adelaide, Aus.)
South Sumatra, Indonesia	*Sesamia inferens* Walker (Lepidoptera: Noctuidae)	Sugarcane	DQ232337	HT_6	K. Muirhead (Univ. Adelaide, Aus.)
Ramu, PNG	*Sesamia grisescens* Walker (Lepidoptera: Noctuidae)	Sugarcane	DQ232316	HT_7	K. Muirhead (Univ. Adelaide, Aus.)
Reunion	*Chilo sacchariphagus*	Sugarcane	DQ232329	HT_6	K. Muirhead (Univ. Adelaide, Aus.)
Giru, Australia	*Bathytricha truncata* Walker (Lepidoptera: Noctuidae)	Sugarcane	DQ232322	HT_8	K. Muirhead (Univ. Adelaide, Aus.)
Bundaberg, Australia	*Bathytricha truncata*	Sugarcane	DQ232323	HT_8	K. Muirhead (Univ. Adelaide, Aus.)
Mackay, Australia	*Bathytricha truncata*	Sugarcane	DQ232333	HT_9	K. Muirhead (Univ. Adelaide, Aus.)
Ethiopia, Girana	*Chilo partellus*	Sugarcane		HT_1	Y. Assefa
Ethiopia, Girana	*Chilo partellus*	Sugarcane		HT_1	Y. Assefa
Somalia	*Chilo partellus*	Maize		HT_1	ICIPE, Kenya
Kenya	*Chilo partellus*	Maize		HT_1	ICIPE, Kenya
Kenya	*Chilo partellus*	Maize		HT_1	ICIPE, Kenya
Mozambique	*Chilo partellus*	Maize		HT_1	ICIPE, Kenya

| Zambia | *Chilo partellus* | Maize | HT_1 | ICIPE, Kenya |
| Tanzania | *Chilo partellus* | Maize | HT_1 | ICIPE, Kenya |

3.4.4   Results

DNA sequencing of the PCR products of African *C. flavipes* specimens yielded eight sequences of length 556 to 669 base pairs (bp). The ends of the alignment were trimmed to exclude those sites for which some specimens were missing data, yielding 556 bp for which all specimens had data. The African sequences were all identical. The sequences were then aligned with 16 sequences from specimens of *C. flavipes* from different parts of the world, and once again the alignment was trimmed to exclude missing data. The final alignment was 419 bp in length, and included 45 variable sites of which 14 sites were parsimony informative.

3.4.4.1 Haplotype network

Nine haplotypes were identified of which five were singletons (i.e. represented by single individuals). The most common haplotype (HT_1; Figure 3.8) was widely distributed in Africa, India and Brazil. The Australian specimens from Mackay, Giru and Bundaberg were different from the rest of the specimens in the study by more than nine mutational steps and formed two closely related haplotypes (HT_8 and HT_9; Figure 3.8) that are not connected to other haplotyes. Two of the three Australian *C. flavipes* specimens in the study were represented in one haplotype (HT_8; Figure 3.8) and were connected to the third specimen from Mackay (HT_9) by one mutational step. The other common haplotype (HT_6; Figure 3.8) was represented in three specimens from the Indian Ocean islands and Indonesia. This haplotype is closely related to one of the unique haplotypes (HT_7; Figure

3.8) that was represented in the specimen from Papua New Guinea. The rest of the

haplotypes (HT_2, HT_3 and HT_4; Figure 3.8) were represented in specimens from North

and South America and Southeast Asia and were closely related to the most common

haplotype (HT_1; Figure 3.8). One of the Southeast Asian specimens from Sri Lanka

formed a unique haplotype (HT_5) that is connected to the haplotype from Jamaica and

South Pakistan (HT_2) by five mutational steps (Figure 3.8).
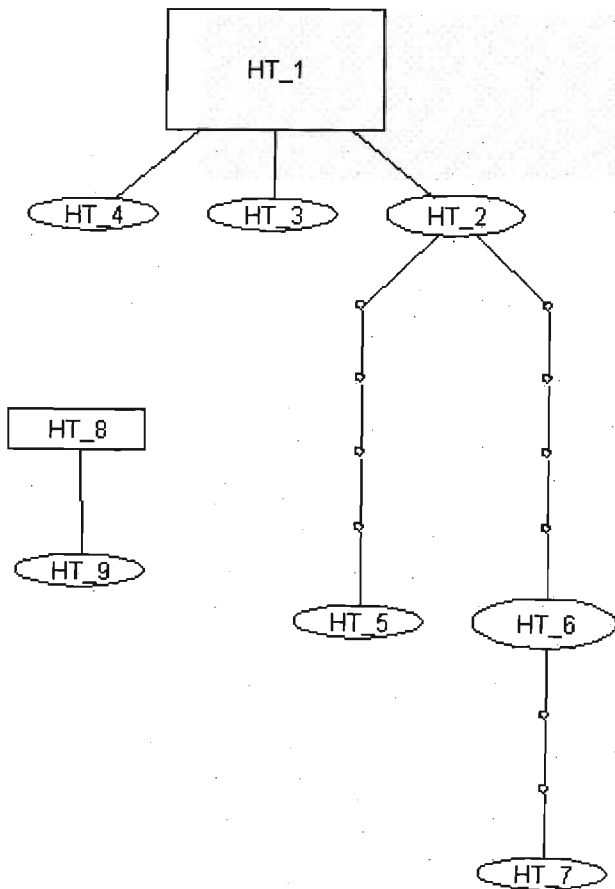
Figure 3.8   Haplotype network showing relationships between the nine haplotypes of *C. flavipes*. Each rectangle/ oval shapes represents a single haplotype, with the size of the rectangle/ oval shapes proportional to the number of individuals within that haplotype. Haplotypes that differed from each other by a single nucleotide mutation are connected by lines. Each small open circle represents one missing haplotype. Haplotype labels are the same as in Table 3.5.

Table 3.6 Percentage uncorrected pairwise distance observed for partial COI DNA sequences within and between clades of *C. flavipes* and *C. glomerata*.

	C. glomerata	Clade I	Clade II	Clade III
Clade I	6.92-7.88	0.00-1.67		
Clade II	7.40-7.88	1.19-3.10	0.00-0.72	
Clade III	7.16-7.40	2.15-3.10	2.39-3.34	0.00-0.24

3.4.4.2 Phylogenetic analysis

Parsimony analysis yielded a single MP tree of 50 steps, with a consistency index of 0.83 and a retention index of 0.94, shown in Figure 3.9. Uncorrected pairwise sequence distances ranged from 0 to 3.34% (Table 3.6). Three clades are apparent. The African sequences, except those from the Mascarene Islands, are included in the first clade together with the sequences from South-East Asian (India, Pakistan, Thailand, Sri Lanka) and North and South American specimens. Divergence reached 1.67% within this clade. This clade has a moderate bootstrap support (73%) and the monophyly of a large subset of these sequences is also moderately supported (74%; Figure 3.9). The second clade is comprised of sequences from the Indian Ocean Mascarene Islands (HT_6), Papua New Guinea (PNG) (HT_7) and Indonesia (HT_6). This clade has strong bootstrap support (96%) and relatively low within-clade sequence divergence (0.72%; Table 3.6). The third clade is exclusively Australian (HT_8 and HT_9). This clade also has strong bootstrap support (98%) and low within-clade divergences (0.24%, Table 3.6).

Results of the molecular analysis evidenced the presence of genetic diversity between *C. flavipes* populations in different geographic regions. However, there was no correlation between host insect and/or plant adapted strains and genetic differentiation.
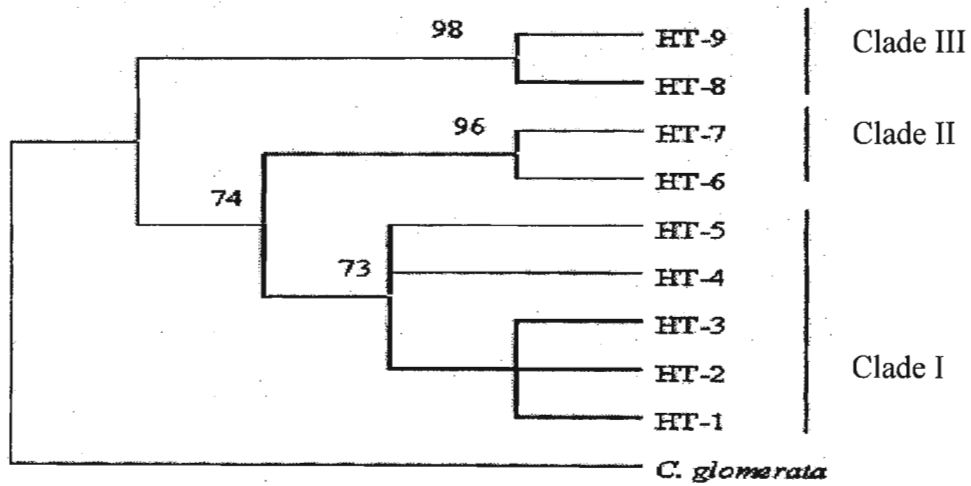
Figure 3.9   Strict consensus tree representing relationships of the nine haplotypes of *C. flavipes*. The tree is rooted with *C. glomerata*. Numbers above internodes are bootstrap support values. Haplotype names are indicated in Figure 3.8 and Table 3.5.

3.4.5 Discussion

This study shows that the African populations of *C. flavipes* sampled from six countries have identical mtDNA fragments. Given the levels of genetic differentiation within the species as a whole, the lack of genetic differentiation among African populations of *C. flavipes* suggests that they have all been derived from the same founding population. The data, therefore, suggests that parasitoids found in Ethiopian sugarcane are derived from the maize/sorghum strain of *C. flavipes* reared by ICIPE and released in different African countries in the 1990s. Our African specimens of *C. flavipes* are closely related to the Indian and Pakistani populations of the parasitoid and significantly different from the populations in the neighbouring Indian Ocean islands, which is congruent with the above hypothesis on the origin of their founding population.

The phylogenetic analysis showed that mtDNA variation in *C. flavipes* is not associated with either host insects or host plants. *Cotesia flavipes* from different hosts were found to be genetically closely related to each other (Table 3.5 and Figure 3.9). This result is surprising and contradictory to previous reports by several authors who suggested the existence of host insect and/or host plant specific strains (Mohyuddin 1971; Mohyuddin *et al.* 1981; Inayatullah 1983; Mohyuddin 1991; Polaszek and Walker 1991; Smith *et al.* 1993; Ngi-Song *et al.* 1995; Ngi-Song *et al.* 1998; Mochiah *et al.* 2001). The data alone does not disprove the existence of host strains because it is possible that mtDNA sequences are not evolving quickly enough to track such host associations, and it is conceivable that more sensitive molecular markers could detect associations not detectable with mtDNA. However, the study does raise the possibility that strains of *C. flavipes* are less host-specific and habitat-specific than previously thought.

The other exceptional result in this study is the high level of dispersal and geographic expansion by this maize/sorghum strain of *C. flavipes* that was recently released in various parts of Africa. In a survey conducted by Omwega *et al.* (1997), *C. flavipes* released in southern Kenya was found in Tanzania about 240 km from the nearest release point four years after release; thus the dispersal rate by this parasitoid was estimated to be 60 km per year. In surveys conducted by Getu *et al.* (2001) in 1999-2000, two years after the release in Somalia, this parasitoid was recovered at many sites in Ethiopia and its establishment in sorghum and maize fields was reported (Getu *et al.* 2003). In the surveys in 2003 and 2004 this maize/sorghum strain of *C. flavipes* was found well established in sugarcane fields (up to 50% parasitism) at Girana, which is more than 2500 km away from the release site in southern Kenya in 1993 (Overholt *et al.* 1994) and more than 2000 km from the release site in Somalia in 1997 (Overholt 1998). As *C. flavipes* has never been released in Ethiopia, the parasitoid must have spread at a rate of more than 200 km per year to become established in sugarcane fields at Girana in Ethiopia. This distance is more than three fold of what was speculated earlier by Omwega *et al.* (1997).

The fast spread and establishment of *C. flavipes* in Ethiopia perhaps can be explained by the cropping system and the abundance of *C. partellus* in lowlands and warmer regions of the country. More than half of the maize and sorghum farmers in Ethiopia practice mixed cropping (Getu *et al.* 2001) and it is not uncommon to see sorghum and/or maize intercropped with sugarcane or planted adjacent to each other surrounded by huge stands of various indigenous wild grasses and sedges. This diversity in host plants undoubtedly has had an impact on the abundance of *C. partellus* that in turn provides a good breeding ground for *C. flavipes*. A review by Overholt *et al.* (1994) and more recent investigations

conducted by Sétamou *et al.* (2005) demonstrated that establishment of *C. flavipes* is greater in places where the most suitable host insect and host plants are abundant. A similar result was reported by Shami and Mohyuddin (1992) when *C. flavipes* exposed to a less preferred host for more than five generations adapted to it and gradually established on it. The role of human activities in the introduction of the parasite can't also be ruled out.

3.4.6 Conclusions

The molecular data indicate that the established *C. flavipes* population in Ethiopian sugarcane on *C. partellus* had its origin from the founding population released in neighboring countries against *C. partellus* in maize. Sorghum/maize strains of *C. flavipes* can thus successfully colonize sugarcane fields at least under Ethiopian cropping systems, but factors responsible for this establishment require further investigation. The rate of spread by the parasitoid is at least three-fold greater than previously reported.

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CHAPTER 4

GENERAL DISCUSSION AND CONCLUSIONS

4.1   INTRODUCTION

In Africa much agriculture is traditional, characterized by smallholdings of no more than one to two hectares per household, labour intensive crop production with little or no external inputs. Crop production takes place under extremely variable and often unfavourable agro-ecological conditions, with annual rainfall ranging from 250 to 750mm in the Sahel in the northwest and in the semi-arid east and south, to 1500 to 4000mm in forest zones in the central west (Abate *et al.* 2000). The productivity of African agriculture is the lowest of all regions of the world and has remained stagnant for many years (Abate *et al.* 2000), especially in terms of graminaceous crops.

Maize and sorghum are among the most widely cultivated cereals in Africa (Ransom *et al.* 1997; ICRISAT 1989). They are considered to be important cereal crops in all developing countries of Africa. Sorghum and maize grains are used for human consumption and brewing (Polaszek and Khan 1998). The stalks of these crops are used for animal feed, fuel, house construction and mulching. Sugarcane, although not a cereal crop, is an important cash crop grown in many parts of Africa (Polaszek and Khan 1998). Its production is a source of employment for millions of people in Africa and it plays a very significant role in the economy of many African nations (SASA 2006, Lichts 2002). Despite the important role of cereal grains and sugarcane as food and cash crops (ICRISAT 1989; Lichts 2002) their yield in Africa remains very low. The average yield of maize and sorghum in Africa remains less than the World average (FAO 2004) and the continent's

sugar production is only about 10 million tonnes, which is less than 10% of the total sugar produced in the World (Lichts 2002).

Several factors are responsible for the low yield of these crops in Africa. The major ones being low soil fertility, drought, weeds, diseases and insect pests. Lepidopteran stem borers are among the key insect pests of cereal grains and sugarcane in Africa (Kfir *et al.* 2002; Leslie 2004; Atachi *et al.* 2005). Most African stem borers are indigenous, and feed inside stems of monocotyledonous plants belonging to the Poaceae, Cyperaceae and Typhaceae (Ingram 1958; Nye 1960; Polaszek 1998).

In a recent survey conducted by Le Rü (unpublished data) in the eastern and southern parts of Africa, 137 species of lepidopteran stem borers have been collected from seventy-five species of indigenous host plants belonging to the Poaceae, Cyperaceae and Typhaceae. However, only eight of these stem-boring species are reported to be economically important pests of cereal crops and sugarcane in Africa (Polaszek 1998; Leslie 2004). The noctuid *Busseola fusca* Fuller (Lepidoptera) and the crambids *Chilo partellus* Swinhoe (Lepidoptera) and *Chilo orichalcociliellus* Strand (Lepidoptera) are known to attack cereal grains in the continent (Ingram 1958; Nye 1960; Polaszek 1998). The crambids *Chilo sacchariphagus* Bojer (Lepidoptera), *Chilo agamemnon* Bles. (Lepidoptera), and the noctuid *Sesamia cretica* Lederer (Lepidoptera) are important only in sugarcane (Leslie 2004). The noctuid *S. calamistis* (Lepidoptera) and the pyralid *Eldana saccharina* Walker (Lepidoptera) are pests of both cereal grains and sugarcane (Polaszek 1998; Leslie 2004).

The pest status of these stem borers and the species composition of the stem borer complex in crop fields varies between regions. Some indigenous stem borers such as *B. fusca* and *E.*

saccharina display significant geographic differences in ecological preference that may be congruent with patterns of molecular variation (see Chapter 3). The genetic structure of these pests is likely to be influenced by their evolutionary history on natural host plants and geographic separation. Despite the high diversity and polyphagous nature of indigenous African stem borers, very little is known about their population genetics and phylogeography (Sezonlin *et al.* 2006).

During the study to expand knowledge on the phylogeography of *E. saccharina* in Africa so that more effective biocontrol can be implemented, surveys in Ethiopian sugarcane in particular revealed a unique stem borer/parasitoid complex. In addition to beginning to unravel the African *E. saccharina* population question, this study was broadened to include studies of *Busseola* species and the natural enemy *Cotesia flavipes* Cameron (Hymenoptera: Braconidae). These studies distinguished two *Busseola* species complex in Ethiopian sugarcane and evaluated the genetic diversity and origin of the introduced parasitoid *C. flavipes* in Africa. This chapter summarizes the ecological and molecular studies presented in the previous chapters, and highlights areas for future research.

4.2   ECOLOGY OF SUGARCANE STEM BORERS IN ETHIOPIA

4.2.1   Diversity of Sugarcane Stem Borers in Ethiopia

While it is well known that lepidopteran stem borers cause serious damage to cereal grains in Ethiopia (Gebre-Amlak 1985, 1988; Getu 2001) little is known of their status in sugarcane that is usually grown close to or mixed with these crops. This is a gross oversight, as sugarcane throughout Africa is part of the gramineous habitat which these

stem borers inhabit. None of the sugarcane stem borers in Ethiopia have been identified scientifically, even to the generic level. Stem borers in sugarcane were simply referred to as "shoot borer", "stem borer" and/or "top borer" based on the crop stage they attacked and the colour and size of the larvae (Haile-Michael 2001).

Current surveys conducted in various parts of the country revealed the presence of four lepidopteran stem borer species on sugarcane: *B. fusca*, *B. phaia*, *C. partellus* and *S. calamistis* (see chapter 2). *Eldana saccharina* was recovered only from indigenous sedges present in swamps bordering Lake Tana, Blue Nile River, Lake Awasa and from sedges growing in irrigation canals of Metehara and Wonji sugar estates.

Three of the four stem borer species recorded in Ethiopian sugarcane in the study are well known pests of cereal grains in various parts of Africa (Harris 1962; Polaszek, 1998), however, only one of these stem borer species, *S. calamistis*, has been described as a pest of sugarcane elsewhere (Polaszek 1998). This is the first time that *B. fusca*, *B. phaia* and *C. partellus* have been reported as serious pests of African sugarcane (see chapter 2). *Busseola fusca* (Polaszek 1998) and *C. partellus* (Charpentier and Mathes 1969; Way and Kfir 1997) were reported to occasionally infest sugarcane; however, *B. phaia* has never been reported from this crop (Nye 1960; Le Rü, unpublished).

Sugarcane all over the world is mostly attacked by local insects that have adopted it as a host consequent to its cultivation, especially on continents (Pemberton and Williams 1969; Conlong 1994; Polaszek 1998). The noctuid *B. phaia* (Chapter 3.2) is another pest that has shifted to sugarcane from its indigenous hosts (Nye 1960) perhaps because of the cultivation of this crop in close proximity to its natural habitat. Important sugarcane stem

borers such as *E. saccharina* are thriving in their natural host plants near sugarcane fields. The risk to Ethiopian sugarcane of being colonized by stem borers such as *E.saccharina* is significant (Mazodze *et al.* 1999). The recent and continuing expansion of agriculture and associated disturbance of the natural environment will expose crops such as sugarcane to greater infestation by indigenous stem borers and enhance their rate of colonization (Conlong, 2001).

4.2.2 Impact of Cropping System and Cultural Practices on Invasion of Sugarcane by Stem Borers

4.2.2.1. Crop diversity

As is common for small-scale farmers throughout Africa, peasant farmers in Ethiopia have long used crop diversity to minimize the risk of crop failure, improve nutrition, increase food security and produce higher yields of particular crops. The practice of increasing crop diversity provides some measure of protection from insect pests when compared to monocultures (Altieri *et al.* 1977; Gold *et al.* 1990) and has been frequently recommended as a way of reducing pest problems in agriculture (Tonhasca and Byrne 1994). However, several reports indicate that increased diversity either had no effect (Cromartie 1975; Lawani 1982) or posed the danger of a build-up of pest populations when adjacent crops, especially of the same plant family, share the same pests (Abate *et al.* 2000). Results from this study of sugarcane fields in Ethiopia support the latter hypothesis. Sugarcane provides the additional benefit to stem borers in that it grows throughout the year, providing a habitat for them, and importantly also, some parasitoids, when maize and sorghum are not

available. Thus it would appear that sugarcane provided an additional host plant resulting in the expansion of the host plant range by known sorghum and maize stem borers.

In peasant farms of Ethiopia, sugarcane is usually grown on small plots surrounded by land planted with sorghum and maize, major hosts of crop damaging stem borers (Gebre-Amlak 1985; Getu *et al*. 2001; Tefera 2004). The commercial estate farms are also located centrally within maize and sorghum growing regions of the country. The practice of growing sugarcane close to or mixed with cereals exposes sugarcane to infestation by these cereal stem borers. In studies on the effects of surrounding crops on the incidence of stem borers in maize, Van den Berg and Rebe (2001) observed larval migration from thin-stemmed forage sorghum to maize. Ndemah *et al*. (2000) also reported that good hosts of stem borer larvae serve as a source of infestation for adjacent crops. Similar results were obtained by Gebre-Amlak (1988) who found that some indigenous grasses growing around maize and sorghum fields were major hosts of *B. fusca* and suggested that they may serve as sources of infestation for crops. The frequent droughts that result in crop failure in Ethiopia may even further enhance colonization of cereal crops and sugarcane by pests. In years where annual crops are devastated by drought, adults of cereal stem-boring lepidopterans will have little alternative but to lay eggs on less favoured plants (Polaszek and Khan 1998), such as sugarcane. Moreover, larval migration from dying stalks of maize, sorghum and wild hosts to sugarcane may occur in years of rain scarcity.

However, and more importantly, the converse is also true with respect to natural enemies, as crop diversification increased the abundance of natural enemies (Abate *et al*. 2000). Results of surveys conducted in Ethiopia also show the significant role of crop diversity in enhancing the establishment of introduced natural enemies (see chapter 3.3).

4.2.2.2 Crop residue management

Crop residue management practices of Ethiopian farmers could also have made a significant contribution to the expansion of host range by these stem borers to include sugarcane. Dry stalks of maize and sorghum are used for building houses and fences, and as fuel and bedding for livestock. They are stacked in fields to dry and left for long periods until used. These crop residues constitute an important reservoir of stem borers that give rise to new infestations in crop host plants growing in the area (Ingram 1958; Gebre-Amlak 1988; Päts 1996). Fully grown larvae of lepidopteran stem borers such as *B. fusca* and *C. partellus* are known to diapause in high altitude areas in the dry season in tunnels made in dry maize and/or sorghum stalks (Gebre-Amlak 1985). As these stem borers are common in maize and sorghum all over Ethiopia (Gebre-Amlak 1985; Getu *et al.* 2001; Tefera 2004), they are most likely the sources of stem borers attacking sugarcane. Non-crop host plants growing in field margins and wetlands around and between sugarcane and cereal crop fields also harbour stem borers and act as reservoirs for these, but also their natural enemies (Bowden 1976; Sampson and Kumar 1986; Conlong 1990; Polaszek and Khan 1998). Old stems of maize and sorghum left standing after harvest may also lead to re-infestation of the crops in the next season (Girling 1978).

Proper sanitation and residue management practices such as burning and ploughing are reported to reduce the carry-over populations and may minimize the chance of the population build-up in sugarcane. However, burning crop residues is not a desirable practice for peasant farmers since dry stalks are used as fodder, building material and fuel (Päts 1996). Therefore, devising alternative sanitation measures such as horizontal

placement of stalks, which exposes the stalks to direct sunlight, (Gebre-Amlak 1988) could be recommended so that the stalks could be used for other purposes.

4.2.3   *Eldana saccharina* **and Ethiopian sugarcane**

In contrast to the pest status attained by indigenous noctuid borers and the exotic crambid *C. partellus* in sugarcane fields of Ethiopia, *E. saccharina* is not a problem for sugarcane production in the country. This borer was recorded from three wetland sedges growing in swampy areas and in the irrigation canals of the sugar estates, however, none of the sugarcane fields were infested by *E. saccharina* (Chapter 2.4). In addition, repeated area wide surveys for lepidopteran stem borers of maize and sorghum conducted in Ethiopia (Gebre-Amlak 1985; Getu *et al.* 2001; Tefera 2004) showed that *E. saccharina* does not attack these crops. It is thus apparent that, currently, *E. saccharina* is not present in commercial and subsistence crop fields. Whether or not it will spread into sugarcane and other cereal crops remains to be seen, but the possibility certainly exists. In Zimbabwe, where the borer was first observed in sedges close to sugarcane in 1987, severe outbreaks in sugarcane by *E. saccharina* were reported from two fields in 1998 (during a severe drought), and the insect has since then spread throughout their industry (Mazodze *et al.* 1999; Mazodze and Conlong 2003). The same could happen in Ethiopia should current biotic and/or abiotic factors change to favor the incursion of *E. saccharina* into sugarcane. Climate, sugarcane expansion (Conlong 1997; Mazodze *et al.* 1999) and agronomic factors such as fertilizer application rates (Carnegie 1981), time of harvest (Nuss *et al.* 1986; Conlong and Mugalula, 2001), and selection of varieties (Nuss *et al.* 1986; Rutherford *et al.* 1993; Bond 1988; Keeping 1999) should continually be monitored in order to predict

the relevant changes in *E. saccharina* densities, and to take corrective action before serious infestation occurs.

Eldana saccharina is known to feed on a wide range of grasses and sedges that undoubtedly differ in their suitability for this stem borer. The insect shows a preference for different hosts in different regions (Conlong 2001). In southern Africa, *E. saccharina* is known to favour wetland sedges in its natural habitat and sugarcane in the subtropical eastern region (Paxton, 1982; Webster *et al.*, 2005). However, a recent survey by Le Rü (unpublished) recovered this insect from an indigenous wetland grass in the North West province of South Africa. The climate in this highveld area reflects temperatures well below the typical mean daily temperatures for sugarcane growing regions of South Africa. This indicates that *E. saccharina* is capable of surviving in colder areas where sugarcane cannot be grown. Surveys for *E. saccharina* therefore need to include highland and cool areas where suitable host plants are available. In areas where this pest is found, farmers should be aware of the danger, and that practices to prevent it from moving into crop fields need to be implemented. Agronomic practices such as high nitrogen fertilizer application and delayed harvesting tend to increase *E. saccharina* populations in crop fields (see Chapter 2.4). These practices thus need to be discouraged, especially in times of drought. The diversity of natural enemies in natural habitats of *E. saccharina* (Conlong 1990, 2000) need to be investigated in time and space, and evaluated for their role in the management of *E. saccharina* in crop fields, as done in South Africa (Conlong 2001).

4.2.4 Indigenous Lepidopteran Stem Borers and Sugarcane

In a recent survey of indigenous host plants of stem borers conducted in various parts of Ethiopia, Le Rü (unpublished) found seven new species of lepidopteran stem borers (see chapter 2). Some of the stem borers reported by Le Rü (unpublished) are recorded only from Ethiopia, indicating variation in the stem borer species complex from region to region and greater diversity of stem boring lepidopterans in Ethiopia than previously thought. By extrapolating from the number of stem borers recorded in the neighbouring Kenya (92 species) (Le Rü unpublished), the number of stem borer species discovered so far in the country is not even a quarter of what is expected to be present. The possibility exists that some of these stem borers may become economically important pests. This is what has happened in many parts of the world where sugarcane is cultivated (Pemberton and Williams 1969). Adaptation of *E. saccharina* to sugarcane subsequent to its extensive cultivation in Zimbabwe (Mazodze *et al.* 1999) and adaptation of *B. phaia* to sugarcane in Ethiopia (Chapter 2.1) are examples of new associations between indigenous stem borers and sugarcane in Africa.

The stem borers infesting indigenous host plants may play a significant role in the population dynamics of parasitoids. Some of the stem borers in the natural habitat may act as alternative hosts for parasitoids leading to augumentation of populations of natural enemies in crop fields (Khan *et al.* 1997; Schulthess *et al.* 1997). However, some borer species may form a reproductive sink by encapsulating the parasitoids (Kfir *et al.* 2002). Accurate identification and knowledge of the stem borers in natural habitats is essential in the design and development of control strategies and prevention of pest incursion. This is

especially important in implementing classical biocontrol for the management of exotic stem borers like *C. partellus* (Zhou *et al.* 2001).

4.2.5 Impact of Indigenous Non-Crop Hosts

Natural habitats are considered to constitute important refuges for lepidopteran stem borers and their natural enemies. Diverse plant species in the Cyperaceae, Poaceae, Typhaceae and Juncaceae (Conlong 2001; Polaszek 1998; Le Rű unpublished) are reported to be the natural homes of stem borers and are assumed to be good sources of natural enemies (Conlong 1997). These indigenous host species could act as trap plants in agricultural situations, to protect crop plants (Khan *et al.* 1997; Van den Berg & Rebe 2001; Midega and Khan 2003) and may provide additional habitat for natural enemies (Conlong 1990; Khan *et al.* 1997; Schulthess *et al.* 1997). Ndemah *et al.* (2001), working in the forest zone of Cameroon, found a higher parasitoid species diversity on *P. purpureum* than on maize. Similarly, Conlong (1997) reported the abundance of natural enemies in indigenous host plants to be much higher than that found on sugarcane. It was suggested that indigenous host plants play an important role in maintaining stable parasitoid populations during the off-season and thereby lower pest incidence in crop fields during the growing season (Ndemah *et al.* 2003).

Currently, information on the diversity and abundance of indigenous host plants of stem borers in Ethiopia is lacking. Further studies on the tritrophic interactions among host plants, stem borers and natural enemies under different local conditions are needed for a better understanding of the role played by indigenous habitats as a source of pests and natural enemies for crop fields.

4.3   MOLECULAR PHYLOGENY AND PHYLOGEOGRAPHY

4.3.1   Phylogeography of *E. saccharina*

The African sugarcane borer, *E. saccharina*, is a polyphagous species that feeds on a variety of host plants (Atkinson 1980; Betbeder-Matibet 1981; Carnegie 1974; Conlong 1997, 2001; Maes 1998; Mazodze and Conlong 2003; Polaszek and Kahn 1998). Studies on such generalist phytophagous insects often reveal that they instead represent complexes of genetically differentiated host races or cryptic species (Martel *et al.* 2003; Stireman *et al.* 2005). Ecological studies on *E. saccharina* have reported the variation in behavior, the natural enemy complex and host plant preferences between populations of the pest in different parts of Africa (Conlong 1994; 2000; 2001; Mazodze and Conlong 2003). In West Africa, *E. saccharina* was reported to favour grasses over sedges in its natural habitat and is mainly a pest of maize (Betbeder-Matibet 1981). In southern Africa, it prefers indigenous wetland sedges and sugarcane (Atkinson 1980). None of the natural enemies recorded in West Africa are found in southern Africa and vice versa (Conlong 2001). In Ethiopia, *E. saccharina* was recovered only from indigenous wetland sedges (Chapter 2.4) but the natural enemy guilds attacking this pest in the country were similar to those reported from West Africa (Conlong 2004 *in lit.*). Conlong (2001) reported that West African and southern African parasitoid populations merged in Uganda. In this part of the continent, *E. saccharina* is known to be a pest of both maize and sugarcane, which is uncommon in West and southern Africa (Girling 1972; Conlong and Mugalula 2001; Matama-Kauma *et al.* 2001). Hence, it was suggested that Uganda could be either a "hybrid zone" where the

different populations of *E. saccharina* make contact or the point of origin from where the different populations of the pest diverged (see Chapter 3.2).

Initial molecular studies on *E. saccharina* (King *et al.* 2002; Assefa *et al.* 2006) supported the hypothesis generated from the ecological studies of Conlong (2001), that there could be different biotypes of *E. saccharina*. A phylogeographic study conducted on the populations of *E. saccharina* from eleven countries in North, East, West and southern Africa revealed the presence of genetic divergence between populations of the pest in different regions (Chapter 3.2). As was the case in the ecological studies (Conlong 2001), the largest portion of genetic diversity revealed in this study is distributed in eastern Africa. High genetic diversity was observed amongst individuals from Kinyara Sugar Works Ltd. in Uganda and individuals from Kenya, with individuals from Kinyara Sugar Works Ltd. distributed through all four groups detected in this study (Chapter 3.2). The Kenyan specimens from the Rift Valley, east of Rift Valley and west of Rift Valley fall into three separate groups (Figures 3.2 and 3.3). Although the Ethiopian specimens were all in one group, there was an indication of genetic sub-structuring in the Ethiopian specimens. Ethiopian specimens from the Rift Valley were different from specimens collected from Lake Tana (Figures 3.2 and 3.3) that is located west of the Rift Valley (Figure 3.1). In contrast to the eastern African specimens, western and southern African specimens from different countries were genetically closer to each other. The relatively high genetic diversity in East African populations of *E. saccharina* as compared to populations from the rest of Africa could be associated with the impact of volcanic eruptions in the Miocene and Pleistocene that significantly altered the hydrology of the region and resulted in the formation of the Rift Valley (Beadle 1974). These geological events probably marked the initial stages of fragmentation of species in the African continent (Hamilton 1982) and could have modified

the population structure of *E. saccharina*. Studies on vertebrate herbivores (Arctander *et al.* 1999; Alpers *et al.* 2004) and on *B. fusca* (Sezonlin *et al.* 2005) have given enough evidence of the impact of these events on the distribution of different animal lineages in Africa. Evidence from this study, however, argues against the existence of host plant races in *E. saccharina*. The first line of evidence for the absence of host plant associated genetic differentiation is the presence of the most common haplotypes on all host plants, in multiple samples, over vast geographic areas (Chapter 3.2). The second line of evidence for absence of host plant-associated lineage is the separation of haplotypes feeding on a specific host plant into different groups (Chapter 3.2).

4.3.2 DNA Barcoding

Prompt identification of a species is critical in framing the correct response to any incursion, forming the basis for appropriate control and eradication measures (Hogg and Hebert 2004). Given that larvae of many species are difficult to separate morphologically and laboratory rearing of larvae to adults for morphological identification can be laborious (Meijerman and Ulenberg, 1998), DNA-based methods could provide a useful alternative (Hogg and Hebert 2004), especially when combined with the phylogenetic methods frequently used for establishing inter- and intra-specific relationships between taxa and within populations (Caterino *et al.* 2000).

DNA sequence variation in a fragment of the COI gene of the mitochondrial genome proved to be useful for discriminating two species of *Busseola* in Ethiopian sugarcane (Chapter 3.2). The DNA-based method was a quick, easy and reliable method. This method may be a solution for situations in Africa where there is an acute shortage of

experts and rearing facilities to keep field collected insects alive until adult emergence for morphological identification. The use of barcoding as a taxonomic tool has been criticized by many authors (Lipscomb *et al.* 2003; Will and Rubinoff 2004; Lee 2004), as it can result in misidentifications, particularly in closely related species. However, it is a good method for organisms when adequate data on their ecology, morphology and molecular systematics are also available for comparison of intraspecific and interspecific variability. The DNA-based methods used in this study were successful because sufficient data on the ecology, morphology and molecular taxonomy of noctuid stem borers was readily available for comparison with molecular data in this study (Moyal unpublished).

4.3.3 Molecular Phylogenetics

One of the fundamental prerequisites for a successful biological control program is the accurate identification of both the target pest and its natural enemies. Success in many programs has been significantly delayed by misidentifications of pests or natural enemies (Holloway 1998). However, morphological identification of closely related groups of species, such as the *Cotesia flavipes* Cameron species complex, is very difficult. The taxonomic history of this parasitoid complex is somewhat confusing due to the difficulty in distinguishing between the species in the complex using external morphology (Alam *et al.* 1972). Morphological identification becomes even more impractical when it comes to the identification of different strains of *C. flavipes*. Molecular systematic analysis has become the commonly used method for examining intra-specific relationships (Evans *et al.* 2000; Scheffer 2000).

Phylogenetic analysis of *C. flavipes* specimens collected from different countries in Africa indicated that the *C. flavipes* established on *C. partellus* in Ethiopian sugarcane most likely had its origin from the founding population released in neighboring countries for the management of *C. partellus* in maize (Overholt 1998). Understanding the genetic similarity between populations of *C. flavipes* from sugarcane in Ethiopia and from maize in other parts of Africa provided information on the adaptation of the sorghum/maize strain of the parasitoid to sugarcane fields (Chapter 3.4). The cropping system practices in Ethiopia, especially the diversity of host plants sharing the same species of stem borer around the fields of sugarcane, is suggested to be the main factor for the rapid establishment of this parasitoid in sugarcane fields of the country (see Chapter 3.4). Overholt *et al.* (1994) and more recent investigations conducted by Sétamou *et al.* (2005), demonstrated that establishment of *C. flavipes* is more successful in places where suitable host insect and host plants are abundant. The establishment of *C. flavipes* in sugarcane fields indicates the potential of the parasitoid as a biocontrol agent against stem borers in sugarcane fields.

4.4   CONCLUSIONS

4.4.1   Ecology of Sugarcane Stem Borers in Ethiopia

The ecological studies conducted in Ethiopia were first aimed at investigating the distribution and importance of *E. saccharina* and collecting sample specimens for the phylogeographic study on this pest. Lack of information on sugarcane stem borers in Ethiopia, however, led the study to be expanded beyond the investigation on the status of *E. saccharina*, to include area-wide surveys to determine ecological aspects of the borer complex in sugarcane and other indigenous host plants in the country. In the surveys, the

host plant range and distribution of *E. saccharina* and other sugarcane borers in Ethiopia were determined and samples of stem borers and parasitoids were collected for phylogenetic and phylogeographic studies.

Quantified area-wide surveys of the sugarcane estates and small-scale farmer fields of Ethiopia verified the presence of four lepidopteran stem borer species on Ethiopian sugarcane. These were *Chilo partellus*, *Sesamia calamistis*, *Busseola fusca* and *Busseola phaia*. *Eldana saccharina* was recovered from large sedges in waterways of Metehara and Wonji sugar estates in the central part of the country, and around lakes in northern and southern Ethiopia.

Understanding the stem borer species in Ethiopia is important to prevent incursion and management of stem borers in crop fields. However, information on the diversity of stem borers in the natural habitat is scarce in Ethiopia. Information on the species diversity of the family Pyralidae, and genera *Manga* (Noctuidae), *Sciomesa* (Noctuidae) and *Torticida* (Tortricidae) is completely lacking in the country and only economically important stem borers in the family Noctuidae and Crambidae were given attention. The role of wild host plants as a reservoir for stem borer infestation and as sources of natural enemies has not yet been studied. In the present study, the occurrence of *E. saccharina* and *B. phaia* in Ethiopia was reported for the first time. This is indicative of the higher diversity of stem borers in the country. More surveys are therefore required for accurate estimation of the stem borer species diversity in Ethiopia, which will make a significant contribution to the management of stem borers in Ethiopia. Data from these surveys could be used to examine changes in the distribution of stem borers, their host plants and their associated natural enemies, and for the planning and execution of stem borer biocontrol and habitat management

approaches. Thus, such studies could make a significant contribution to the management of stem borers in Ethiopia.

4.4.2 Molecular Phylogeny and Phylogeography

4.4.2.1   Phylogeography of E. saccharina

Results of the current study show the existence of considerable genetic diversity among populations of *E. saccharina* from different parts of Africa. The main drivers of this genetic diversity are believed to be the tectonic movements of the earth and resulting volcanic eruptions in eastern Africa during late Miocene and early Pleistocene, and the subsequent re-establishment of habitat linkages during the late Pleistocene. The eastern Rift Valley seems to act as a geographic barrier to gene flow between *E. saccharina* populations. Uganda was suggested to be a hot spot and/or a melting pot for *E. saccharina* populations in Africa. No host plant associated genetic differentiation was observed.

4.4.2.2 Studies on Busseola *and* C. flavipes

Sequence divergence in the mitochondrial cytochrome-*c* oxidase I (COI) gene was used as a tool to identify the species of *Busseola* attacking Ethiopian sugarcane. Partial sequences from the COI region of the mitochondrial DNA of Ethiopian specimens were compared with sequences of identified adult specimens of noctuid species from the East African region. Results of the sequence analysis indicated that the *Busseola* species complex in Ethiopian sugarcane comprised *B. fusca* and *B. phaia*.

Partial sequences from the COI region of the mitochondrial DNA were also used to investigate the origin of the parasitoid *C. flavipes* in Ethiopian sugarcane. Results of the analysis revealed that the *C. flavipes* population that had established in sugarcane fields of Ethiopia was genetically similar to the populations released against *C. partellus* in maize in other parts of Africa.

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