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Host plant diversity of *Sesamia calamistis*: cytochrome *b* gene sequences reveal local genetic differentiation

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Abstract

Sesamia calamistis Hampson (Lepidoptera: Noctuidae) is one of the indigenous stem borer pests associated with maize (*Zea mays* L.) and sorghum [*Sorghum bicolor* (L.) Moench] (both Poaceae) in Africa. Its pest status varies across the continent and this has been attributed to variation in diet breadth and ecological preferences among populations. Its larvae were found on 12 plant species during a study initiated at four sites (Muhaka, Mtito Andei, Kakamega, and Suam) in Kenya to estimate its diet breadth and genetic population structure. Ten of the infested plant species belonged to the family Poaceae [*Echinochloa haploclada* (Stapf) Stapf, *Eleusine corocana* L., *Eleusine jaegeri* Pilg., *Panicum deustum* Thunb, *Panicum maximum* Jacquin, *Pennisetum purpureum* Schumacher, *Setaria verticillata* (L.) P. Beauv., *Sorghum arundinaceum* (Desvaux) Stapf, *S. bicolor*, and *Z. mays*]; the other two were Cyperaceae: *Cyperus distans* L. and *Cyperus dives* Delile. Combined with collections from other African countries (Uganda, South Africa, Benin, Ghana, Nigeria, and Togo), comparisons of partial cytochrome *b* sequences revealed the presence of 68 haplotypes that differentiated into clades I and II. In Kenya, the two clades colonized different regions, except in Mtito Andei where they co-existed. Individuals from Mtito Andei could be separated based on their host plants: clade I with 14 haplotypes was found mainly on maize (78.6%), whereas clade II with 10 haplotypes was found mainly among wild host plants (63.6%). Detection of divergence among these clades with cytochrome *b* suggests that their evolutionary separation may have taken place about one million years ago. This article discusses the potential implication of this differentiation for the management of *S. calamistis* as a pest of maize and sorghum in Africa.

Introduction

Sesamia calamistis Hampson (Lepidoptera: Noctuidae) is one of the indigenous stem borer pests associated with maize (*Zea mays* L.) and sorghum [*Sorghum bicolor* (L.) Moench] (both Poaceae) in Africa (Ingram, 1958; Bowden, 1976). However, its economic importance varies across the continent. It is a major pest in West Africa, but remains a minor pest in eastern and southern Africa (Moyal, 1988; Bosque-Pérez & Schulthess, 1998). Differences in its pest status may be attributed to variations in diet breadth and ecological preferences among populations (Kfir, 1997; Seshu Reddy, 1998). *Sesamia calamistis* is reported to have originally colonized non-cultivated hosts belonging to the Poaceae and Cyperaceae (Harris, 1962) and presumably switched to cultivated crops after the domestication of sorghum and the introduction of maize in Africa (Polaszek & Khan, 1998). In an attempt to reduce losses associated with *S. calamistis* infestation, efforts have been made to understand its ecology and the importance of wild host plants in its population build-up (Gounou & Schulthess, 2004; Le Rü et al., 2006a; Ong'amo et al., 2006). Like other phytophagous insects, adaptation to feed on sorghum and maize may have been accompanied by physiological and

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behavioural changes through a process of natural selection (Futuyma & Moreno, 1988). As host choice and oviposition behaviour among phytophagous insects are genetically determined (Jaenike, 1990), natural selection may have favoured oviposition on hosts that supported growth and survival of offspring (Gassmann et al., 2006).

Host selection may result in the evolution of genotypes suited for different host plants (Futuyma & Moreno, 1988; Jaenike, 1990), which may in turn affect economic importance and spatial distribution of phytophagous pests. Previous studies show that economic importance of *S. calamistis* varies across Africa (Schulthess et al., 1997). In the Kenyan context, similar variations in economic importance are observed along varying altitudinal gradients among cultivated crops (Ong'amo et al., 2006). High densities are observed in low altitude areas and low densities in high altitude areas. However, the *S. calamistis* management initiatives in Africa generally ignore the possible existence of genetic variability among populations in different regions. In addition, an understanding of the ecological factors that govern density variation across regions is lacking. This study was initiated in Kenya to investigate genetic variability among *S. calamistis* populations in sites with different insect densities. The mitochondrial cytochrome *b* gene was chosen as marker, because it has been used in a phylogeographic study of another noctuid, *Busseola fusca* (Fuller), in Africa (Sezonlin et al., 2006).

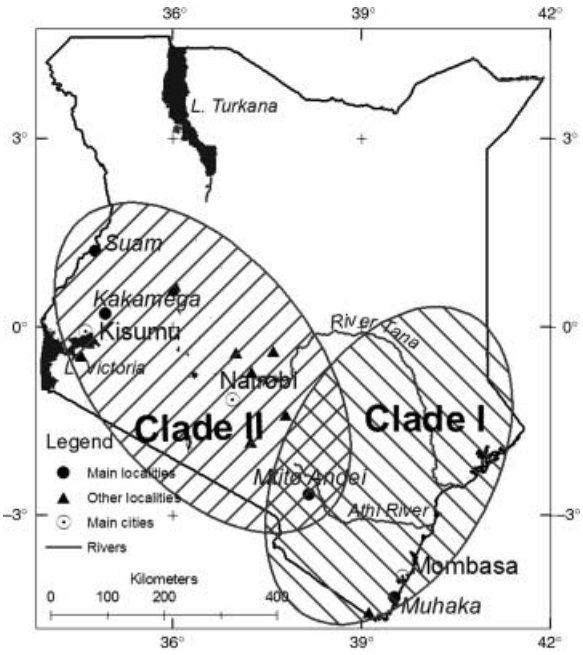

Figure 1 Map of Kenya showing the four study sites and the estimated spatial distribution of *Sesamia calamistis* clades I and II. Estimates on spatial distribution of the two clades were based on findings of the current study and results of the ongoing phylogeography study in Africa.

Materials and methods

Survey sites and processing of specimens

Stem borers were collected from four sites (Suam, Kakamega, Mtito Andei, and Muhaka; Figure 1) across different vegetation mosaics in Kenya (White, 1983). These sites represent regions characterized by different growing seasons and stem borer pest composition (Ong'amo et al., 2006). To capture these variations, two surveys were undertaken in each of the sites during cropping and non-cropping seasons and the results were pooled (Table 1). Within each study site (ca. 25 km^2), cultivated fields were randomly chosen, and the number of fields per site was in proportion to the total cultivated area. Plants growing in the cultivated fields (*Z. mays*, *S. bicolor*, and *Eleusine corocana* L.) and wild habitats (wild host plants) in the neighbourhood were inspected for stem borer infestations. During each survey, plants with stem borer infestation symptoms were dissected in situ for larval recovery, plants were identified, and larvae were kept separately with respect to host plant species and site. *Sesamia calamistis* larvae are morphologically similar to some other *Sesamia* and *Sciomesa* species and their identity can only be confirmed in the adult stage, based on the genitalia. Therefore, all recovered larvae were reared to the pupal stage on artificial diet, as described by Onyango & Ochieng'-Odero (1994). Pupae were kept in separate plastic vials until emergence of the moths. Upon emergence, the moths were identified and preserved in absolute ethanol (>99%) until DNA extraction.

DNA extraction and sequence analysis

Total genomic DNA was extracted from the thoracic muscles using a commercial kit (DNeasy™ Tissue Kit; Qiagen, Hilden, Germany) protocol with proteinase K digestion as recommended for animal tissues. The extracted DNA was stored at –20 °C until required for amplification. Voucher specimens are housed at the ICIPE–Biosystematics Unit, Kenya. Polymerase chain reaction (PCR) was used to amplify the 873-bp cytochrome *b* mitochondrial fragment using the primers CP1 (5′-GATGATGAAATTTTGGATC-3′) (modified from Harry et al., 1998) and Tser (5′-TATTTC-TTTATTATGTTTTCAAAAC-3′) (Simon et al., 1994). The PCR was performed on a Biometra GeneAmp PCR System in a 25-µl reaction mixture containing 1 µl of the genomic DNA, 5× Green GoTaq® Flexi Buffer, 0.24 mM dNTPs, 3 mM $MgCl_2$, 0.4 µM of each primer, and 1 unit of *Taq* polymerase (GoTaq; Promega, Mannheim, Germany). After initial denaturation at 94 °C for 5 min, PCR conditions

Infested plants species	Individuals processed			
	Muhaka	Mtito Andei	Kakamega	Suam
Cyperus distans	–	2	–	–
Cyperus dives	–	–	–	2
Echinochloa haploclada	1	–	–	–
Eleusine corocana	1	6	2	4
*Eleusine jaegeri**	–	2	–	–
Panicum deustum	–	1	–	–
Panicum maximum	2	1	–	–
Pennisetum purpureum	–	3	1	–
*Setaria verticillata**	–	1	–	–
Sorghum arundinaceum	3	11	–	–
Sorghum bicolor	14	4	2	–
Zea mays	24	47	31	1

Table 1 List of plant species from which *Sesamia calamistis* larvae were collected of the four study sites in Kenya. Species followed by an asterisk represent new host plants of *S. calamistis*

were 40 cycles of 94 °C for 1 min of denaturation, 46 °C for 1.5 min of annealing, 72 °C for 1.5 min of extension, and a final extension period of 10 min at 72 °C. The PCR products were visualized by means of electrophoresis in 1% agarose gel previously stained with ethidium bromide to verify amplification. Amplified products were purified with the Promega Wizard SV Gel and PCR Clean up System following the manufacturer's protocol. DNA sequencing reactions were performed using an ABI PRISM® BigDye™ Terminator v3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems, Darmstadt, Germany), cleaned using ethanol/EDTA precipitation. Sequences were visualized on an ABI 3130 automated sequencer using Big-Dye fluorescent terminators. The consensus sequences obtained were aligned manually using MacClade 4.05 (Maddison & Maddison, 2001). Additional sequences of individuals from maize (P Moyal, unpubl.) were compared with collections from other localities in Kenya and some African countries (South Africa, Uganda, Benin, Ghana, Nigeria, and Togo). All sequences were deposited in GenBank (accession numbers EU305065–EU305228).

Evaluation of reproductive parameters

Fourth and fifth instars of *S. calamistis* were collected from sorghum in Kisumu (western Kenya) and from Cyperaceae species in Shimba Hills (eastern Kenya, coastal region). Larvae of both populations were reared separately on artificial diet as described by Onyango & Ochieng'-Odero (1994) until pupation. Some randomly picked individuals from each population were sequenced at the cytochrome *b* gene for the population genetic analysis. Male and female pupae were kept in separate plastic boxes (30 × 12 × 10 cm) containing a moist cotton pad to maintain about 80% r.h., and monitored for adult emergence. Pupae and adults were maintained in a controlled chamber at 25.3 ± 0.9 °C, 68.6 ± 12.8% r.h. (mean ± SE), and under reversed L12:D12 photoperiod with the scotophase from 07:00 to 19:00 hours, hereafter referred to as night. The reversed photoperiod allowed all experiments to be carried out during the day.

In each population, 1-day-old males and females (minimum 10 individuals of each sex per night) were released in a net cage (40 × 40 × 63 cm) at the onset of the night. They were provided with a diluted honey solution on a piece of cotton wool. Mating behaviour was observed and recorded every 30 min during the scotophase. Mated pairs were transferred from the cage to a transparent plastic jar (16 cm high × 9 cm in diameter), to facilitate the assessment of the duration of copulation. The plastic jar contained a wet piece of cotton wool that maintained the r.h. at around 80%. One cylindrical surrogate stem made from a rectangular piece of nylon cloth (15 cm long × 5 cm wide) rolled helicoidally from top to bottom was placed in each jar. This support had earlier been found to elicit good ovipositional response in *S. calamistis* (P.-A. Calatayud, personal observation). The eggs laid by each female were counted each night, and the surrogate stem was renewed every night throughout a female's life. Life duration and the time of emergence of the first neonate after egg-laying were recorded for each female.

Statistical analysis

Basic sequence statistics were calculated using DnaSP (Rozas et al., 2003). The following parameters were used to estimate genetic variability among populations between sites (Muhaka, Mtito Andei, Kakamega, and Suam) and between host plants in Mtito Andei (wild and cultivated hosts): number of haplotypes (h), number of polymorphic sites (S), haplotype diversity (d) (Nei, 1987), nucleotide diversity (π) (Lynch & Crease, 1990) using the Jukes and Cantor correction (Jukes & Cantor, 1969), and mean number

of nucleotide differences (K) (Tajima, 1983). Analysis of the extent of genetic differentiation between the populations (F_{ST}) (Hudson et al., 1992) was performed with the Arlequin 2.000 software (Schneider et al., 2000). A maximum parsimony network was drawn using TCS 1.21 software (Clement et al., 2000). For reproductive parameters, means were computed and separated by Mann–Whitney U-test. Rank analysis for a two-sample test was performed using Statview software, version 5.0 (Abacus Concepts, Berkeley, CA, USA).

Results

Diet breadth of *Sesamia calamistis*

Sesamia calamistis larvae were sampled from 12 plant species (Table 1). For the purpose of this study, all plant species from which *S. calamistis* larvae were recovered have been considered as host plants without quantifying their relative contribution to *S. calamistis* population dynamics. The host list also contains previously unknown hosts (Khan et al., 1997; Gounou & Schulthess, 2004; and Le Rü et al., 2006b), with their importance varying among surveyed geographic sites. In addition to the cultivated cereals (maize, sorghum, and finger millet), larvae were found on *Cyperus dives* Delile and *Sorghum arundinaceum* (Desvaux) Stapf in Suam, and on *Pennisetum purpureum* Schumacher in Kakamega. Together with the cultivated cereals, the larvae were recovered from seven more host plants in Mtito Andei (*Cyperus distans* L., *Eleusine jaegeri* Pilg., *Panicum deustum* Thunb, *Panicum maximum* Jacquin, *P. purpureum*, *Setaria verticillata* (L.) P. Beauv. and *S. arundinaceum*) and three in Muhaka (*Echinochloa haploclada* (Stapf) Stapf, *P. maximum*, and *S. arundinaceum*) (Table 1).

Differentiation of *Sesamia calamistis* populations in Kenya

The TCS maximum parsimony network of 195 sequences revealed 68 haplotypes. These haplotypes separated into two clades with an average divergence of $1.89 \pm 0.24\%$ (Figure 2). Clade 1, containing 33 haplotypes, showed an average divergence of $0.36 \pm 0.19\%$, whereas clade II, containing 35 haplotypes, showed an average divergence of $0.44 \pm 0.20\%$. Except for three specimens (GenBank accessions EU305074, EU305088, and EU305112), all other individuals from Muhaka and part of the collection from Mtito Andei grouped in clade I, together with individuals from South Africa. Individuals from Suam, Kakamega, and the others collected from Mtito Andei grouped in clade II, together with individuals from Uganda, Benin, Togo, Ghana, and Nigeria.

The spatial distribution of the two clades in Kenya varied: clade I was mainly found in Muhaka and Mtito Andei, whereas clade II was found in Mtito Andei, Kakamega, and Suam. The partial distribution of individuals from Mtito Andei into clade I and II suggested greater genetic variability in that area. This was further reflected in the genetic diversity parameters (S, h, d, π, and K) that revealed higher variability in Mtito Andei than in Muhaka, Kakamega, and Suam (Table 2A).

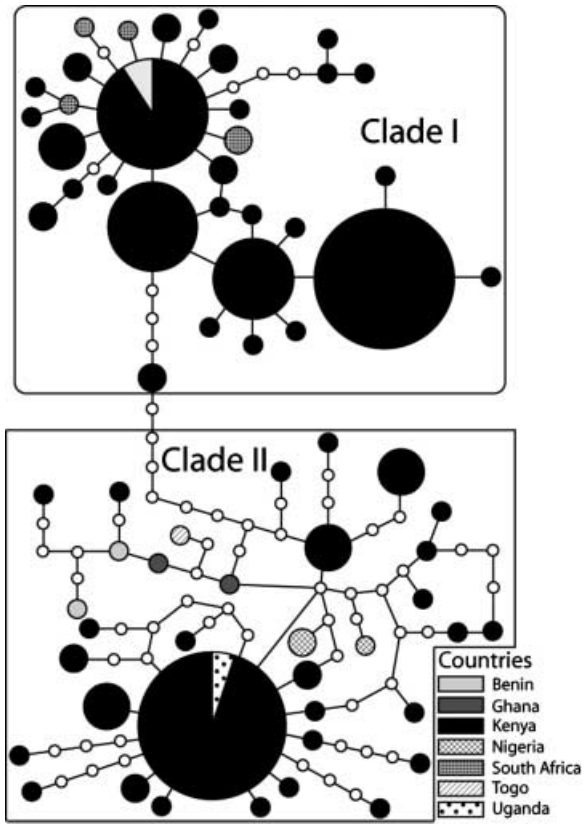

Figure 2 TCS haplotype network of 195 *Sesamia calamistis* specimens processed from seven African countries. Lines represent the most-parsimonious relationships between haplotypes, shaded circles represent individual haplotypes proportional to the number of samples sharing a haplotype, and unshaded nodes indicate inferred steps not found in the samples. Different shading patterns represent different countries.

Genetic differentiation in host utilization (Mtito Andei)

Sesamia calamistis was found on a wide range of cultivated and wild host plants in Mtito Andei. Values obtained from genetic diversity parameters (d, π, and K; Table 2B) indicate that individuals from the wild plants are genetically more variable than individuals from the cultivated hosts. However, there were more haplotypes among cultivated hosts (h = 16) than among wild host plants (h = 13). Excerpt of haplotypes in Mtito Andei from the global network (Figure 2) indicated that individuals in the respective clades varied in terms of host plant preference, suggesting differentiation with respect to host plants ($F_{ST} = 0.4008$;

Table 2A Genetic diversity (mean ± SD) of the cytochrome *b* gene in *Sesamia calamistis* populations from four localities in Kenya

	Sampled sites			
Genetic diversity parameters	Muhaka	Mtito Andei	Kakamega	Suam
No. sequences (n)	43	78	35	8
No. segregating sites (S)	30	35	23	11
No. haplotypes (h)	15	24	12	5
Haplotype diversity (d)	0.792 ± 0.060	0.834 ± 0.034	0.677 ± 0.082	0.786 ± 0.151
Nucleotide diversity (with Jukes & Cantor correction) (π)	0.004 ± 0.001	0.009 ± 0.001	0.003 ± 0.001	0.004 ± 0.001
Mean no. nucleotide differences (K)	3.508 ± 1.822	7.740 ± 3.644	2.927 ± 1.563	3.214 ± 1.852

Table 2B Genetic diversity (mean ± SD) of the cytochrome *b* gene in *Sesamia calamistis* populations from wild and cultivated hosts in Mtito Andei

	Mtito Andei	
Genetic diversity parameters	Cultivated host	Wild hosts
No. sequences (n)	57	21
No. segregating sites (S)	30	25
No. haplotypes (h)	16	13
Haplotype diversity (d)	0.723 ± 0.076	0.891 ± 0.049
Nucleotide diversity (with Jukes & Cantor correction) (π)	0.006 ± 0.001	0.010 ± 0.001
Mean no. nucleotide differences (K)	5.312 ± 2.603	8.381 ± 4.073

P<0.001). The first group with 14 haplotypes corresponded to clade I, while the second group with 10 haplotypes corresponded to clade II (Figure 3). Most individuals in clade I (87.5%) came from cultivated host plants (*Z. mays*, 78.6%; *E. corocana*, 5.4%; and *S. bicolor*, 3.5%), whereas clade II was dominated by individuals mainly from wild host plants (63.6%). However, haplotypes of individuals from *S. arundinaceum* appeared in both clade I and II, accounting for 7 and 32%, respectively.

Reproductive and life trait parameters

Clade I and II populations appeared to vary in the start of mating after onset of the scotophase (Table 3; Mann–Whitney U-test: U = 150, P<0.0001); mean mating times of clade I and II populations were 5.4 and 7.1 h, respectively. This variation was attributed to differences in the female calling hours observed during the study (data not included). There were also differences in mating duration (Mann–Whitney U-test: U = 51.5, P = 0.04) with clade I taking longer (129.6 min) than clade II (99.6 min). Despite the variation in mating time and duration, all females oviposited exclusively during the scotophase. There were no differences between the two populations in lifetime egg production (Mann–Whitney U-test: U = 169.5, P = 0.79) or the time of first eclosion (Mann–Whitney U-test: U = 182, P = 0.64). Each female laid about 680 eggs and the eggs took about 8 days before eclosion. On average, however, the females of clade II lived slightly longer

Table 3 Reproductive parameters (mean ± SE; sample size in parentheses) of the clade I and II populations of *Sesamia calamistis* as recorded under laboratory conditions

Biological parameters		Hour of mating (h after the onset of night)	Mating duration (min)	Total no. eggs laid per female	Female life duration (days)	Time of eclosion after egg laying (days)
Populations	Clade I	5.4 ± 0.2 (37)	129.6 ± 8.5 (16)	685.0 ± 47.8 (21)	6.9 ± 0.3 (23)	8.1 ± 0.2 (23)
	Clade II	7.1 ± 0.1 (32)	99.6 ± 13.8 (12)	679.5 ± 39.2 (17)	7.7 ± 0.2 (19)	8.1 ± 0.1 (19)
Statistics[1]	U	150	51.5	169.5	87.5	182
	P-value	<0.0001	0.04	0.79	<0.01	0.64

[1]Mann-Whitney U-test (rank analysis for two-sample test).

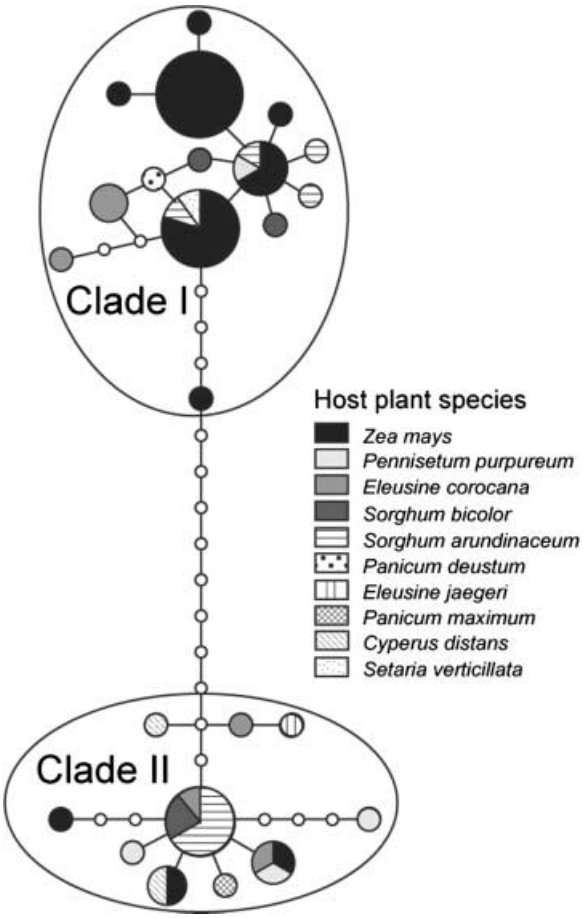

Figure 3 TCS haplotype network of *Sesamia calamistis* individuals collected from 10 host plant species in Mtito Andei. The area of each circle is proportional to the number of samples in that haplotype. Lines represent the most-parsimonious relationships between haplotypes, shaded circles represent individual haplotypes, and unshaded nodes indicate inferred steps not found in the samples. Different shading patterns represent the different host plants.

(7.7 days) than those of clade I (6.9 days) (Mann–Whitney U-test: $U = 875$, $P = 0.009$).

Discussion

This study showed that populations of *S. calamistis* in Kenya are divided into two clades: clade I dominant in the southeast and clade II in the southwest, and that the two clades co-exist in central Kenya. The genetic distance between both clades (about 1.8%) suggests an ancient divergence. Assuming that the mitochondrial substitution rate of 2.3×10^{-8}/site/year for arthropods in general (Brower, 1994) also applies here, diversification may have occurred about one million years ago. Individuals collected from other localities in Kenya confirmed the observed geographic differentiation. Collections from Uganda, Ghana, Benin, Togo, Nigeria, and South Africa showed a similar pattern: clade I was found only in South Africa, clade II only in Uganda, Nigeria, Benin, Togo, and Ghana – all to the west of Kenya. From a more general African perspective, the clades can be classified as east (clade I) and west (clade II), the two meeting in central Kenya.

Currently, *S. calamistis* appears to thrive on maize and sorghum, though it has retained a close association with its original host plants. Larvae of both clades were found on cultivated as well as wild host plants in all four localities in Kenya. Preference for maize and cultivated sorghum is reflected in the higher densities in the cultivated fields, as has been observed earlier (Ong'amo et al., 2006). Shanower et al. (1993) noted the same phenomenon under experimental conditions and attributed low numbers of larvae among wild host plants to their poor nutritive value. Evolution of host and/or oviposition choice by *S. calamistis*, like other phytophagous insects, may be based on allelochemicals and/or the quantity and quality of plant nutritive compounds (Jaenike, 1990). Interactions between these factors may favour oviposition on suboptimal host plants. This may ultimately lead to variation in performance and survival of progeny on different hosts, as observed by Gassmann et al. (2006) during their study on adaptation of *Ophraella notulata* (Fabricius) to feed on *Ambrosia artemisiifolia* L.

Unlike in other localities that were dominated by individuals from either clade I (Muhaka) or II (Kakamega and Suam), there was evidence of genetic differentiation with respect to host plant use in Mtito Andei, where both clades co-existed. In this locality, almost all individuals belonging to clade I were found on maize, whereas most individuals of clade II were found on wild plants. However, some individuals of clade I were found on wild plants and this explained the high genetic variability observed in this clade. Differentiation of these populations with respect to host plants is not limited to *S. calamistis*; similar results have been recorded for another noctuid, the fall armyworm, *Spodoptera frugiperda* (Smith). Two populations of *S. frugiperda* inhabiting the same geographical area showed patterns of differentiation, including the formation of host races (Prowell et al., 2004). The observed separation among *S. calamistis* populations in host use in Mtito Andei could be attributed to either low attraction of maize to clade II or competitive advantage of clade I on that host plant.

Clade II could be considered as a population that recently started to invade cultivated fields after having retained wild grasses as preferred hosts in the expansive Kakamega (Kenya) and Mabira (Uganda) forests for a long time. Even though there is no evidence to support this hypothesis,

retaining wild plants as their preferred hosts may have been facilitated by low agricultural activities around Mabira forest (Uganda) and Kakamega forest (Kenya). These forests and their environs are colonized by a wide range of natural enemies associated with diverse noctuid stem borers that inhabit the forests (BP Le Rü, unpubl.) and this may have limited rapid population build-up and subsequent expansion in this area. In addition, other noctuid stem borers, such as *B. fusca* (Fuller), may out compete *S. calamistis* in cultivated fields, as they oviposit early in the season, whereas *S. calamistis* moths arrive later and show little preference regarding the infestation level of their host plants (Seshu Reddy, 1983). This may explain the observed low *S. calamistis* densities in Kakamega (clade II) compared to Mtito Andei (clade I). Perhaps competition excluded clade II from the maize fields in Mtito Andei, where both clades exist. Although Shanower et al. (1993) did not test performance of the two *S. calamistis* clades on different host plants, they observed variations in the development time among different hosts. Because of the high nutritive value of maize plants, stem borers reared on the latter complete their development faster than individuals reared on wild host plants. In Mtito Andei, clade I is mainly found on maize plants and probably completes its development well before clade II, re-infesting the available hosts (both maize and wild plants) before emergence of moths of clade II. This would exclude clade II from the cultivated fields and limit its population to the few available wild host plants. Under this scenario, interbreeding between the two clades would be reduced strongly.

Applied entomologists across Africa are presently concerned with *S. calamistis* because of its pest status (Bosque-Pérez & Schulthess, 1998; Gounou & Schulthess, 2004; Le Rü et al., 2006a). The finding of – potentially 1-million-year-old – geographical differentiation of two clades may help explain the variation in pest status observed across Africa (Seshu Reddy, 1983; Bosque-Pérez & Schulthess, 1998), and it may radically influence management initiatives. For sustainable management of *S. calamistis*, a region-specific approach based on knowledge of the dominant clade needs to be adopted. However, several questions with practical implications may still be asked. For example, has clade I, which constitutes an important stem borer proportion in low altitude areas in Kenya, adapted fully to the new host plants (maize and sorghum)? Is the observed preference (rather than an expanded host range) evolutionarily favoured because of trade-offs in fitness on different plants, with adaptations to the new host reducing fitness on the original host (Futuyma & Moreno, 1988)? Further laboratory and field studies need to be done at other sites and in different geographical locations. Only in this way can the spatial pattern of host plant colonization be better understood.

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