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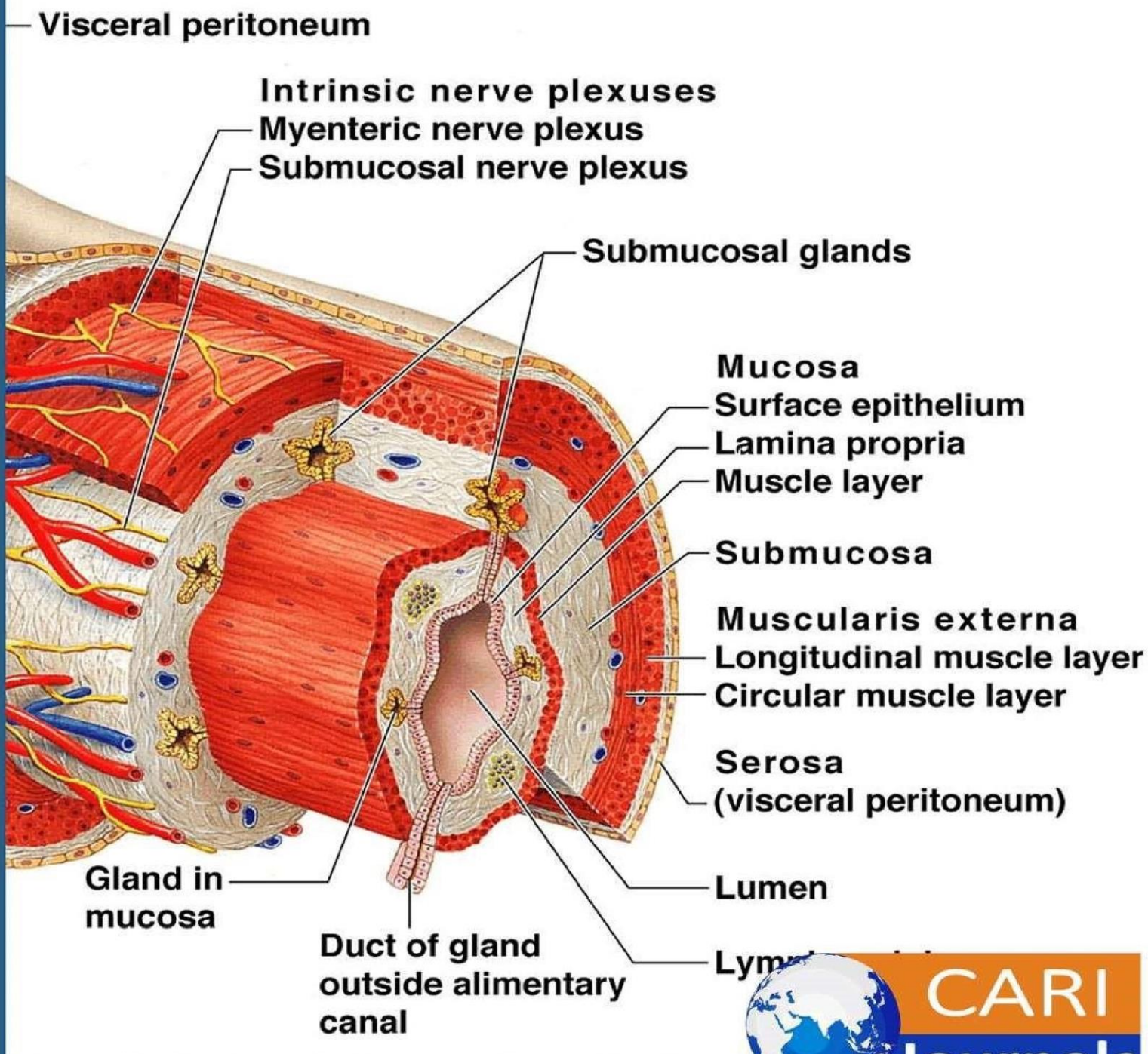

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Morphological and Molecular Characterization of Fall Armyworm, *Spodoptera Frugiperda,* From Selected Regions in Kenya.

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Abstract

Purpose: This study was done in Kenya for the purpose of confirmation and tentative identification of fall armyworm to help in proper monitoring and effective management of the pest. To achieve this, the study was organized in order to characterize *Spodoptera frugiperda* (fall army worm) found in Kenya using morphological and molecular techniques and determine prevalent strain of *Spodoptera frugiperda* in eastern and central regions of Kenya. The study was also organized to compare the host diversity for *Spodoptera frugiperda* strains in eastern and central regions of Kenya.

Methodology: The study was a cross-sectional study which was conducted in 5 regions namely; Machakos, Nyeri, Murang'a, Embu and Kiambu. Sampling of sub counties was done followed by random choice of at least 2 villages and eventually reaching the actual households. Questionnaires were used to interrogate farmers about their knowledge on fall armyworm and seeking permission to check and pick the fall armyworm from their farms. Samples of moths and larvae were obtained. The pest was identified morphologically in the field before being taken to the lab for DNA was extraction and COI gene amplification. The amplified DNA was shipped to Macrogen, Netherlands for sequencing.

Findings: This study confirmed actual establishment of *Spodoptera frugiperda* in eastern and central Kenya using COI gene amplification and analysis. Phylogenetic analysis indicated the presence of both the "Rice" and "Corn" strains. Results indicated higher prevalence of the Rice strain at 77.8% while that of Corn strain was 22.2%. Investigation of the host plants for the fall armyworm gave no evidence of plant host specificity for R- strain since it was also found in *Zea mays* (maize). Only two plants species, maize and sorghum, were found to host *Spodoptera frugiperda* in the study region, with a higher preference towards the maize crop.

Unique Contribution to Theory, Policy and Practice: The findings of morphological and molecular characterization together with phylogenetic studies confirms presence of rice and corn strains of *Spodoptera frugiperda* in Kenya. The pest was recorded mainly in maize and sorghum crop and due to its host range in its native Western Hemisphere and migration ability it may spread to other crops like millet, rice, cotton, vegetables etc. Host status should be continuously investigated. It was also found that *Spodoptera frugiperda* has established itself in central and eastern regions of Kenya meaning that permanent solution to control its effects have to be developed.

Keywords: *Spodoptera Frugiperda, Strain, Corn, Rice*

Introduction

General Background

Fall armyworm (*Spodoptera frugiperda*) is a Lepidopteran pest whose larvae feeds on leaves, stems, inside whorls of young maize plants and damages many other plant species, causing major damage especially to other cultivated crops in the glass family like rice, sorghum and sugarcane (State Department of Agriculture [SDA], 2018). Severe outbreak of the pest was first reported in several African countries in 2016 (Nagoshi *et al.,* 2019). It entered Kenya through Uganda and was first reported in March 2017 in western Kenya (world vision international, 2017). There is no absolute documented information about its origin but it's suspected to have entered Kenya via shipment of imports from America or China. The pest is suspected to have spread rapidly over a period of two years to maize-growing areas in the country (De Groote *et al.,* 2020). In these maize growing regions, farmers had started using traditional methods like application of soil, ash and sand to suffocate the worms. However, KARLO recommended scouting for early identification of infestation and physical killing of the pest. Gichere *et al.,* (2022) did susceptibility of fall armyworm to insecticides which included; spinetoram and spinosad (Spinosyns), lambda cyhalothrin and deltamethrin (Pyrethroids), lufenuron (Benzoylurea) and found out that the spinosyns were more lethal to fall armyworm than pyrethroids and benzoylurea.

Problem statement

There are two sub populations of fall armyworm; C- Strain which is associated with corn, sorghum and cotton, and R – strain associated with rice, alfalfa, pasture grasses and millet (Murua *et al*., 2015). Morphological and behavioral similarities between the strains pose a challenge in their identification leaving molecular methods as the most accurate methods to identify and differentiate the two (Katrina *et al*., 2021). Morphological identification should be confirmed by DNA analysis in order to categorize the fall armyworm into their respective sub groups. Such information is important in the management of the pest especially when designing toxins which can be used in their control. The pest has been in Kenya since 2016 and information about its host diversity is limited only to maize and rice hence the need investigate availability of other hosts.

Literature Review

Fall army worm (*Spodoptera frugiperda*)

Fall army worm is a Lepidopteran pest whose larvae feeds on leaves, stems, inside whorls of young maize plants and damages many other plant species. It belongs to the family Noctuidae and the order Lepidoptera. The female lays eggs in masses of 150-200 and it can lay up to 2000 eggs in 30 days which are spherical, 0.4 to 0.75 mm in diameter, initially green and become light brown prior to exclusion. They mature in 2-3 days at 20-30^{o}C (Sarkowi, F. N., & Mokhtar, A. S. (2021). There are usually six larval instars. The head capsules widths are about 0.35 mm, 0.45 mm, 0.75 mm, 1.3 mm, 2.0 mm and 2.6 mm respectively. The lengths of the instars 1 to 6 are 1.7, 3.5, 6.4, 10.0, 17.2 and 34.2 mm respectively. Larva 1 is white to yellow with black head capsule. Larva 2 and 3 darken as they feed and prior to moulting to the next instars, larva 4 to 6. Larva 4-6 have varying colour

depending on what they feed. They have dark coloured head capsule with a white inverted" Y". They have four dark spots arranged in a square on top of the eighth abdominal segment, the last segment (Assefa, F., & Ayalew, D. (2019).

Origin fall armyworm into Kenya

Severe outbreaks of fall armyworm were first reported in several African countries in 2016. It was detected in central and western Africa and was later reported to have spread to sub-sahara African countries (Goergen *et al*., 2016). It is suspected to have entered Kenya from Uganda. Fall armyworm was first reported in western Kenya in March 2017 where it had infested counties of Busia, Trans-Nzoia, bugoma, Uasin-Gishu and Nandi. This was confirmed by Kenya Plant Health Inspectorate Service and Kenya Agricultural and Livestock Research Organization.

Impact of fall armyworm on crops in Kenya

Fall armyworm damages crops like maize, rice, sorghum, pear millet, napier grass and many other crops - thus it's a polyphagous pest (Michael *et al.,* 2018). This results to economic loss. Attack of crops has caused negative economic impact and food insecurity due to the crop damage. Currently the pest has quarantine status in Europe and thus has negative implications on exports from Kenya (Kenya Agricultural and Livestock Research Organization [KARLO], 2016).

Genetic level advantages to fall armyworm survival and strain differences

Genetic level advantages to fall armyworm survival

The major challenge is predicting the severity of infestation at migratory destination by fall armyworm. This is due to genetic heterogeneity within the species of fall armyworm which increase phenotypic variability. Genetic and physiological differences between FAW host strains presents limitation to hybridization between the strains of the pest (Meagher *et al*., 2008).Liu *et al.* (2019) analysed fall armyworm genome at chromosome level and found out that there is expansion of cytochrome p450 and glutathione 5-transferase gene families which are functionary related to detoxification and pesticide tolerance. This means that the pest has capability of resisting pesticides.

Genetic difference identified in *Spodoptera frugiperda* strains

The strains of *Spodoptera frugiperda* are morphologically identical, but can be distinguished using DNA barcodes which show two distinct clusters that may have diverged 2 myr ago and now have a mean sequence divergence of 2.09% (Cock *et al.,* 2017).

The genome was sequenced in 2014 (GeneBank accession is GCA_002213285.1) and it's in form of scarfords. Gouin *et al*., (2017) analyzed whole genome sequences from laboratory and natural populations of both strains. They observed expansions of genes associated with chemosensation and detoxification. They also found that C strain has genome size of 438 Mb while R strain has 371 Mb. They also noted that C strain genome contains 21,700 predicted protein coding genes while R strain has 26,329. The C strain was found to have 59 CYPs numerous enough as they are involved in insecticide resistance. CYP6, CYP9, CYP321 and CYP324 were expanded. CYP9A91

was specific to R strain. In C strain, genes associated with chemosensation, digestion and immunity were overrepresented while in R strain, genes involved in detoxification and digestion were overrepresented (Gouin *et al.*, 2017). Based on mtCOI gene, site mtCOI1164D is used for strain identity with T_{1164} identifying the rice-strain *COI*-RS haplotype group and either A_{1164} or G_{1164} indentifying the corn-strain *COI*-CS l (C_{1164} has not been found in this species). Polymorphisms at five other sites (mCOI1125Y, mCOI1176Y, mCOI1182Y, mCOI1197R, mCOI1216W) have been used to categorise *Spodoptera frugiperda* based on hemisphere (Meagher *et al.*, 2019). Nagoshi *et al*., (2012) identified four major haplotypes on C strain based on COI gene i.e., h1 (A1164 A1287), h2 (A1164 G1287), h3 (G1164 A1287 and h4 (G1164 G1287).

Control and management of FAW

Control and management of FAW in America – Western hemisphere

In America, insecticides are applied to maize in South Eastern U.S to control FAW. The insecticides used are categorized into high risk and low risk insecticides. The high-risk insecticides used contain restricted active ingridients and examples include lonnate, lorsban, asana, perm-up, baythroid, declare, bifenture, hero, warrior, mustang maxx, tombstone avaunt and besiege. Low risk insecticides include coragen, premio, voliam targo, minecto pro, belt, black hawk entrust, radiant, match, intrepid etc. Both categories are available in Africa. In U.S, spinetoran, indoxacarb and chlorantra niliprode are effective in controlling order larvae while novaluron, lufenuron, difflubenzuron and methoxyfenozide can control early to mid-stage larvae. However, studies have indicated that after continued use the pest starts developing resistance (Plasanna *et al.*, 2018). Organic farmers in America are advised to use Bt sprays formulated as crystal protein, crystal protein with spores or entire bacteria.

In mexico, brazil and parts of N. America maize resistance to FAW has also been used. This kind of maize is yet to be developed in Africa. Bt maize hybrid are grown in brazil and North America (Burtet *et al.*, 2017). Egg parasitoids are also used in America to augment the biological control of *Spodoptera frugiperda*. The main parasitoid used is *Telenomus remus* (Harrison *et al*., 2019).

Control and management of FAW in Kenya – Eastern hemisphere

KALRO (2018) reports that measures have been taken to control and manage FAW. They include; authorization of pesticides for interim use and efficacy evaluation. Examples include Abamectin+chlorantraniliprole, Lufenuron, Emamectin benzoate, Pyriproxyfen and Acephate. KARLO harnessed technical expertise to guide the management of FAW from Brazil. Launching of nationwide control flat form was done. This included training and demonstrations on control to farmers, providing pesticides and personal protective equipment for emergency use and bringing knowledge and technical gaps by focusing on status and socioeconomics of fall armyworm, insecticides effectiveness and application regimes and reporting the response, biopesticides, entomopathogenic isolates and botanical extracts. In 2017 the ministry of agriculture and irrigation adopted rapid response to FAW by capacity building, surveillance and awareness creation. The

ministry advises farmers to intercrop maize with legumes and do scouting to ensure timely actions if damage or signs are noted.

Materials and methods

Study site

The study was conducted in 5 regions namely; Machakos, Murang'a, Kiambu, Embu and Nyeri counties, with the following coordinates: Machakos (1º32'54.0744''S, 37º12'56.5848''E), Nyeri (0º34'54.4176''S,36º55'43.3668''E), Murang'a (0º43'45.9768''S, 37º9'3.69''E), Embu (0º27'41.5764''S,37º42'22.896''E) and Kiambu ((1º05'86.34864''S, 36º47'40.58'E). These regions were selected because of the cultivation of the documented host plants which is practiced in the areas. More information was collected from the farmers by use of questionnaire. Both larvae and moths in all identified fields were placed in small translucent plastic containers containing 90% ethanol. They were sealed and labeled with the relevant information. The larvae were morphologically identified by presence of four black spots from the second to the last segment. On the dorsal side, eighth segment, the four black spots were arranged in square form (Navasero *et al.,* 2019). Presence of inverted 'Y' shaped yellow band along the fronto -cypeal suture and ecdysial line was also used to identify the larvae (Bhavani, B., *et al*., 2019). Identification of adult moths was guided by the study of Goergen *et al*., 2016. Both larvae and moths of FAW were blotted between two papers to remove excess ethanol. They were rinsed with 1% NOAH for a minute, then with 1x phosphate buffered saline. After airdrying, each of the specimen was placed in 1.5ml microcentrifuge tube and homogenized under liquid nitrogen by using plastic motors and pestle.DNA was extracted according to the method of Asghar *et al.,* 2015. The crushed sample was treated with proteinase K before lysing using lysis buffer and eventual DNA extraction using isolate II Genomic DNA kit (Bioline, UK) following the manufacturer's instructions. DNA quality was checked using a nano Drop spectrophotometer and visualization on a 1% agarose gel Gel red (Babu *et al.*, 2019). Bromophenol blue was used for monitoring the DNA through the gel. Electrophoresis of the DNA in polymerized gel was done at 50V for 45 minutes. Cytochrome oxidase subunit I, COI gene amplification was done using published primers targeting COI genes; COI891F, 5'T ACACGAGCATATTTTACATC3' as forward primer and COI1472R, 5'GCTGGTGGTAATTTTGATATC3' as the reverse primer (Nagoshi *et al.,* 2019). The PCR amplification was done using thermal cycler (**Brand of cyder**) in 30µl reaction volumes. One reaction volume contained 19.2µl of nuclease free water, 6µl of my taq buffer, 0.6µl of reverse primer, 0.6µl of forward primer, 0.6µl of my taq enzyme and 3µl of template. The PCR mix was placed in the thermal cycler set for initial denaturation at 95ºC for one minute, denaturation at 95ºC for 15 seconds, annealing at 47ºC for fifteen seconds, extension at 72ºC for thirty seconds, final extension at 72ºC for seven minutes in a total of 35 cycles and final hold at 4ºC (infinite time). In each reaction a non-template negative control was used (PCR water). Out of 30µl of the PCR product obtained 5µl was ran on 1% agarose gel against a 1kb molecular marker to check the product size. The expected product size was 603bp (Nagoshi *et al.,* 2019). The product was

visualized under a U.V Gel Documentation System. The PCR product was aliquoted and set for shipping to Macrogen Europe for sanger sequencing.

Genetic Diversity and Phylogeny

Multiple alignment of mtCOI was performed using MUSCLE hosted in geneious software. The consensus sequences were extracted and then blasted in the NCBI. The alignments were saved in mega format using Mega 11 software (Tamura *et al.,* 2021) which was then used to construct phylogenetic tree using the neighbour joining method, NJM. NJM was implemented in MEGA 11 software. 1000 bootstrap replicates were used to test for robustness of the phylogeny. The model used was maximum composite likelihood model (Gichui *et al.,* 2020). The reference sequences were obtained from gene bank, including ten sequences from R strain (Accession numbers; MT779914, MT779897, MT779896, MT779852, MT779838, MT779832, MT779828, MT779821, MT779816 and MT779815—From Eastern Hemisphere, China) and six sequences from C strain (Accession numbers: AY714300, AY714298, AY714299- From Northern hemisphere, USA, KF624877 and KF624876 – From Northern Hemisphere, Brazil and MK295625 from Northern Hemisphere, India.). Population descriptive statistics were assessed using DnaSP software version 6.12.03 (Nayyar *et al.,* 2021). These included nucleotide diversity (π), number of haplotypes (H), haplotype diversity (Hd) and genetic neutrality tests such as Fu & Li's D* and F*, Tajima's D and Fu's F. These statistics were computed for each of the study regions. Population structure of the sampled regions was studied by performing analysis of molecular variance (AMOVA) based on F_{ST}. These haplotype frequencies were computed using Arlequin (Wang, *et al.,* 2020) v.3.5.2.2 software.

In order to unravel the biogeographic patterning and the genetic diversity based on their geographic regions, the study populations were divided into five broad groups of Nyeri, Muranga, Machakos, Kiambu and Embu. The percentage of observed variance within and between groups was calculated for determining the genetic molecular variations. Significance was obtained by 1000 permutations tests.

Host diversity

The fields within the five study regions where *Spodoptera frugiperda* had been found were randomly selected for surveying. Maize and crops with vegetative parts planted near maize were sampled using a 'zigzag' W pattern (Baudron *et al.,* 2019). The survey was categorized into two based on the probability of finding *Spodoptera frugiperda* as reported from the literature review. The first method was applied on individual crops which are reportedly preferred by *Spodoptera frugiperda, namely* Maize, Rice, Sorghum and cotton. The second method involved surveying and recording presence or absence of the *Spodoptera frugiperda* on the visited fields. A total of about 500 solitary plants and 180 fields were surveyed in different farms. The survey was carried out in the morning and evening hours.

RESULTS

Field samples collected

There was a total of 49 *Spodoptera frugiperda* samples collected from the five (5) sampled counties of Kenya. Majority of these were larvae with instar ranging between 3-6**.**

Morphological identification of fall armyworm larvae and moths

The first instar larvae were characterized by black head and whitish body (Bhavani *et al.,* 2019). The second instar larvae had brown head and the body segments appeared to conform to color of their current food as guided by Bhavani *et al.,* 2019. The fourth to the sixth instars had a reddish-brown head, raised dark spots with bear spines, and white subdorsal and lateral lines on the body. They had an inverted Y shape on the head and several lines traversing along the segments as observed by Lestar *et al.,* 2020. They were arranged in trapezoid on the first seven abdominal segments and arranged in a square on the second from last abdominal segment (Navasero *et al.,* 2019). The moths had the following characteristics as identified by university of Minesouri, 2020: male moths had light to dark fore wings while the female had grayish-brown mottled forewings with light and dark splotches. Both male and female mature moths had a distinctive white spot near the apex of each forewing, which was more pronounced in the male.

Molecular identification of fall army warm based on the CO1 gene

DNA of *Spodoptera frugiperda* amplified using specific primers which targeted a section of COI gene and subjected to gel electrophoresis was visualized under ultraviolet (UV) light (Fig 3). It showed product size of approximately 600-bp. This was the expected product size based on the primers used (Nagoshi *et al*., 2019). The sequencing results as received from Macrogen (Macrogen (Netherlands), Amsterdam) were compared with GenBank sequences and the homology ranged between 97%-100%. This sequence-based homology confirmed that the samples and

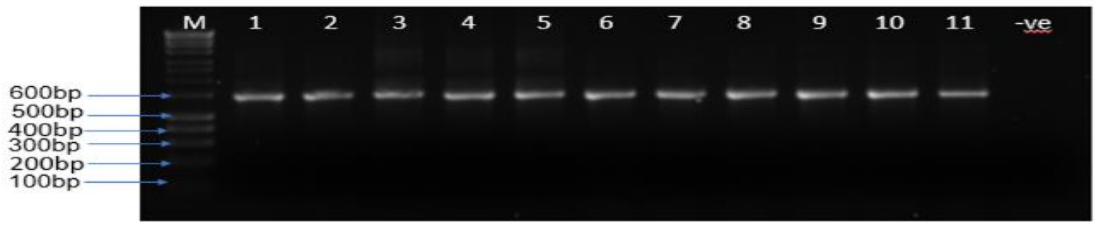

Fig.3 Showing gel electrophoresis of the PCR product. To ascertain the quantity of the product, 100 base pair gene ruler M was used. The product size was about 600 base pairs**.**

Phylogenetic analysis

Sequences from the *Spodoptera frugiperda* samples collected from the study regions were analyzed and used to construct a phylogenetic tree against relevant sequences obtained from GenBank database (Fig 4). The results showed that there were two groups of fall armyworm of which 39 (79.592%) specimens clustered with the COI-rice strain while 10 (20.408%) specimens clustered with the COI-corn strain. *Spodoptera exigua* was included as an out group.

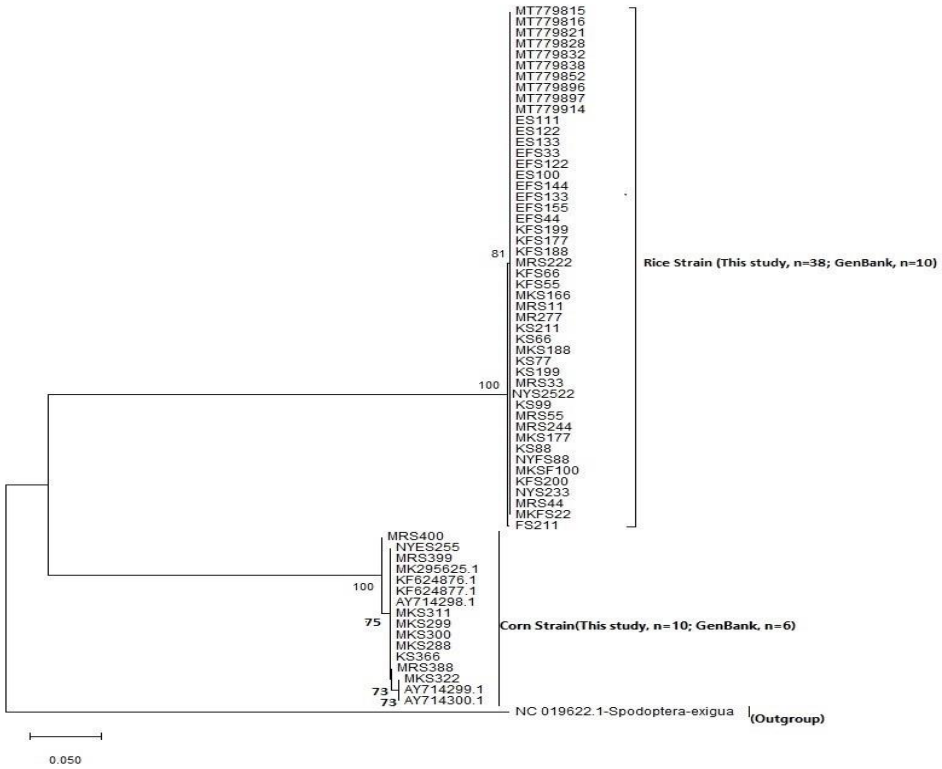

Fig 4:

Phylogenetic tree of *Spodoptera frugiperda* for R-strain samples (38 from east and central Kenya and 10 from GeneBank) and C strain samples (10 samples from east and central Kenya and 6 from GenBank). Sequences from samples are labelled according to the region they were collected (NY-Nyeri, E-Embu, MRS-Murang'a, MKS/MKFS-Machakos, KFS-Kiambu) while those from GenBank are labelled by their accession numbers. Bootstrap values are indicated below the branches. A sequence of *Spodoptera exigua* is included as an out-group. The evolutionary history was inferred using the Neighbour Joining Method.

Prevalence of *Spodoptera frugiperda* strains

From the samples that were sequenced, NCBI blast results showed eight of the samples belonged to other species mainly *Mythmna loyeri*. In Machakos, 12 samples were analyzed and **58.3%** were R strain while **41.7%** were C strain. In Murang'a, 10 samples were analyzed out of which **70%** were found to be R strain while 30% were C of strain. The 10 (100%) samples collected and analyzed from Embu were found to belong to R strain. Out of the 4 samples from Nyeri analyzed, 75% were of R strain while 25% were found to be of C strain. In Kiambu, 92.3 % of the 13 samples collected and analyzed were of R strain while 17.7 % was C strain. Overall, out of the 49 samples analyzed, 79.592% belonged to R strain while 20.408% belonged to C strain.

DNA divergence between the two populations of *Spodoptera frugiperda*

This study investigated 65 mtCO1 gene sequences. The sequences were analyzed by test of phylogeny using MEGA 11 hosted in Genious software followed by polymorphic studies using DNaSP, POPART and Arlequin softwares. Forty-nine sequences were from the sampling population while sixteen sequences had been retrieved from GenBank, of which 6 sequences were of C-strain and 10 sequences were of R-strain. Ten (20.41%) of the sequences from the sampling population were identified as *Spodoptera frugiperda* C-strain while 39 (79.59 %) of sequences were identified as *Spodoptera frugiperda* R-strain. Analytical studies of the study sample *Spodoptera frugiperda* using DnaSP v.6.12.03 revealed presence of DNA divergence between the two populations (C-strain population and R-strain population) (Table 1). The number of fixed DNA differences between the populations was zero. Mutations found to be polymorphic in population 1 but monomorphic in population 2 were 7 while mutations that were polymorphic in population 2 but monomorphic in population 1 were 6**4**

Table 1 Showing DNA divergence between C-strain population and R-strain population

	Population 1 (C-strain)	**Population 2 (R-strain)**
No. of polymorphic sites	93	124
Average nucleotide difference	19.578	14.127
Nucleotide diversity Pi	0.09279	0.06695

Note: The diversity on the nucleotides was noted to be silent (Pi- silent) while the silent mutations were 96.00

Out of the mutations identified in this study, 89 were shared between the two populations while 96.0 were silent. In both populations, nucleotide diversity Pi, was 0.17390. The average number of nucleotide differences for the two populations (k) was 36.692.

Gene flow and genetic differentiation between the two populations was also performed using DnaSp at 1000 replicates. The analysis revealed 6 haplotypes in population of C strain and 15 haplotypes in the population of R strain from the samples collected from eastern and central Kenya**.** The haplotype (gene) diversity (Hd) for C strain was 0.77778 while that of R strains was 0.78718 indicating two distinct haplotypes from their COI gene sequences. As it is evident here, majority of COI sequences which were investigated belonged to R strain (n=39) and they showed no particular specific distribution pattern within the five counties. There was no region-specific haplotype in the five regions.

The total data estimated in gene flow and genetic differentiation showed a higher number of haplotypes, h, which was equivalent to 19 with haplotype diversity of 0.85388. The distribution of the 19 haplotypes as obtained from data file was as shown in the table 2**.** There was no region-specific haplotype within the five counties. They all showed an irregular distribution pattern throughout the five samples counties.

Table 2. Haplotype diversity of *Spodoptera frugiperda* populations from five counties in Kenya (Machakos, Muranga, Kiambu, Nyeri and Embu) studied using mtCOI gene.

Haplotype	No. of samples/sequences	Sample region
1	1	Nyeri
2	2	Machakos, Murang'a
3	5	Kiambu (1), Murang'a (2), Machakos (2)
4-5	1	Machakos
6	1	Murang'a
7-10	1	Embu
11	2	Embu (1), Machakos (1)
12	1	Nyeri
13	2	Embu (1), Murang'a (1)
14	1	Kiambu
15	8	Kiambu (4), Machakos (1), Murang'a (2), Embu (1)
16	1	Embu
17-18	1	Kiambu
19	17	Embu (2), Kiambu (5) Machakos (4), Murang'a (3), Nyeri (2),

Note: Haplotypes 1-5 all belong to R-strain while the rest belong to C-strain.

Population genetic structure analysis

Variation in the population genetic structure was noted to be significant (P <0.001). Major variation was noted to be due to differences between individuals within populations. This percentage variability between individuals was 89.08% while the variability among populations was 10.92 % (Table 3).

Table 3 AMOVA weighted average over Loci results of haplotyping partial mtCOI gene. Sequences were clustered according to the counties of collection to form population.

Source of Variation	Sum of squares	Variance Components	Percentage variation	P-value
Among populations	26.067	0.33718	10.91652	0.00000
Within populations	151.333	2.75152	89.08348	
Total	**177.400**	**3.08870**		

The correlation of the genetic variability among the entire sampling populations of this study showed that Nyeri population had a relatively high variation genetically from the other four (4) populations of Embu, Muranga, Kiambu and Machakos. Moreover, Embu population indicated homogeneity with Murang'a, Kiambu and Machakos populations (Fig 5). Also, Murang'a population varied slightly from Kiambu and Machakos populations (Fig 5). A slight genetic variability was also noted between Kiambu and Machakos (between 0 and 0.2) populations.

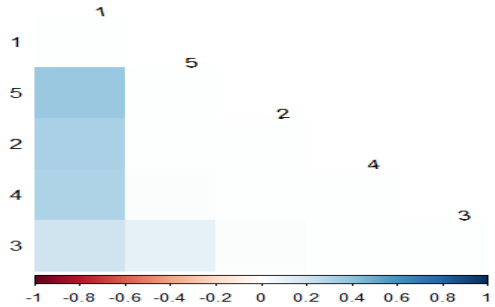

Fig.5. Showing a correlation plot representing genetic variability of *Spodoptera frugiperda* among the entire sampling populations: Nyeri **(1),** Muranga **(2),** Machakos **(3),** Kiambu **(4)** and Embu **(5)**

Population size changes

From the mtCOI sequences of the sample of fall armyworm collected from the 5 counties in Kenya the pairwise number of differences for population size changes curve indicated neutral evolution in the population as the curve was bimodal (Fig.6)

Genetic diversity of FAW populations analyzed on the basis of partial mtCOI gene from five

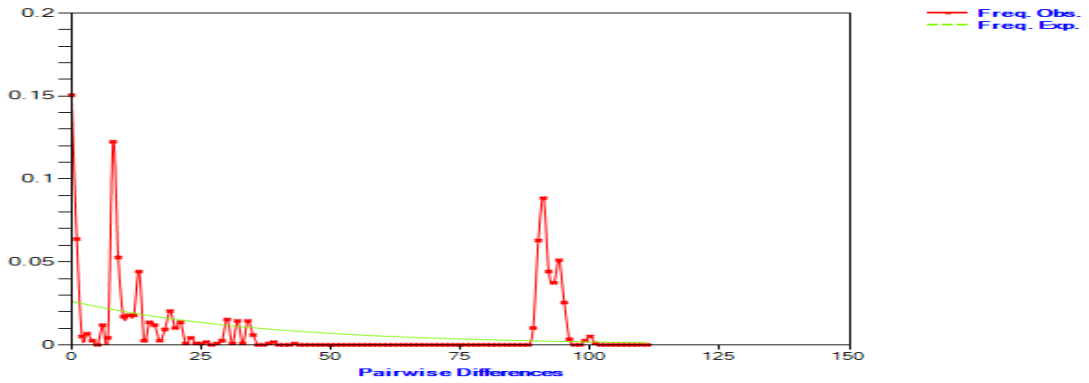

Fig 6. The mtCO1 mismatch distribution bimodal curve showing the observed (solid red line) and expected (dotted green line) pairwise nucleotide site Divergences. This was constructed with DnaSP. Y axis represents relative frequency

Table 4. Genetic diversity of *Spodoptera frugiperda* populations from five regions (counties) in Kenya- Machakos, Murang'a, Kiambu, Nyeri and Embu studied using the partial **mtCOI** gene

	Machakos,	**Murang'a,**	**Kiambu,**	**Nyeri**	**Embu**	**Total**
No. of Sequences (n)	11	11	13	4	10	**49**
No. of sites	436	436	436	436	436	
Sequence homozygosity	0.772	0.836	0.944	1.00	0.947	**0.852**
Polymorphic sites (S)	125	131	118	16	62	**127**
Segregating sites (s)	125	131	115	16		
No. of mutations	155	145	121	16	75	**160**
No. of haplotypes (h)	8	5	6	2	7	**19**

counties in Kenya (Machakos, Muranga, Kiambu, Nyeri, Embu) was summarized below (Table 4). The values of Fu's F statistics were all positive in the population of fall armyworm from all

Haplotype diversity (Hd)	0.894	0.844	0.795	0.078	0.911	**0.849**
Nucleotide diversity (π)	0.2771	0.22583	0.07932	0.03354	0.08137	**0.17539**
Fu's Fs statistic	6.311	11.746	7.134	_	2.904	**10.602**
Fu and Li's D* test statistic	-0.27048	0.93345	-2.78582	_	-0.92462	**0.49429**
Fu and Li's F* test statistic	-0.11442	1.02699	-2.97466	_	-1.00073	**0.31872**
Tajima's D	0.37423	0.83386	-2.08586	_	-0.76898	**0.11287**

counties. The Fu and Li's D* and F* statistics were all negative except in Murang'a while Tajimas D was positive overall (0.11287). n, number of sequences analysed; s, number of segregating sites; h, number of haplotypes; Hd, haplotype diversity (± SD); π, nucleotide diversity; D* and F*, Fu and Li's statistics (Fu and Li 1993); D Tajima's statistic (Tajima 1989). (** indicate statistical significance at 5%, NS=not significant).

Host Diversity of FAW

The results of this study found only *Zea mays* (maize) and *Sorghum bicolor* (sorghum) to be host to FAW from the selected regions in Kenya despite surveying more than 20 different plants. The other plants surveyed in the study included; *Gossypium herbaceum* (Cotton), ,*Oryza sativa (*Rice), *Pisum sativum* (Green peas), *Ipomoea batatas* (sweet potato), *Capsicum annuum* (common pepper), *Solanum lycopersicum* (Tomato), *Pennisetum purpureum* (Napier grass), *Pisum sativum* (Peas), *Phaseolus vulgaris* (French beans), *Phaseolus vulgaris* (Common beans), *Capsicum annuum* (Sweet pepper), *Brassica oleracea v.*, (Colewort), *Brassica oleracea v. capitata* (Cabbage), *Manihot esculenta* (Cassava), *Ilex paraguariensis* (Amaranth), *Spinacia oleracea* (Spinach), *Citrullus lanatus* (water melon), *helianthus annuus* (Sunflower), *Allium cepa* (Onion), *Saccharum officinarum* (Sugarcane) and *Solanum tuberosum* (Irish potato). Although this study was carried out throughout the year, information on various host plants remains limited as only 5 counties were surveyed.

Nature of damage caused by *Spodoptera frugiperda* to the host

Sampling and scouting showed that *Spodoptera frugiperda* was present in farms sampled in the five regions and caused damage characterized by various symptoms including translucent

membranes which occurred in patches. Where these patches occurred, only 1st and 2nd instar larvae were present. Pin holes were also noticed as symptoms. Small, medium and large holes on the leaves as well as damage to developing maize cobs were also observed, mainly attributed to late instar (**4th** to **6th**) larvae. Large quantities of Fras were also found where there was severe damage on crops.

DISCUSSSION

This study reports on the genetic structure, diversity, distribution, existence and preference of corn and rice strains of fall armyworm in eastern and central regions of Kenya. This study identified the two strains in Eastern and Central regions of Kenya based on mitochondrial COI gene polymorphisms, with 79.59% of the samples belonging to the R - strain and 21.41% to the C - strain. Similar dominance trend of the R-strain was also previously documented in Uganda (Omuut, *et al.*, 2023), India (Nayyar *et al.*, 2021) and other parts of Africa and Asia (Acharya *et al*., 2021). They were collected from maize. This can be interpreted to mean that the association of *Spodoptera frugiperda* with its hosts is not absolute. Nagoshi *et al*., (2009) also reported that there was no evidence of statistically significant correlation with maize acreage, a situation supported by this study as even smaller farms (less than one acre) were observed to be infested by this pest.

The pest could even be more spread to other regions in Kenya as its movement is likely to be by short flights in more variable directions to adjacent favorable habitats rather than the long-distance wind-directed migration as observed in North America by Nagoshi *et al.*, 2019. The population neutrality genetic test statistics, based on the mtCOI has revealed that populations of *Spodoptera frugiperda* in Kenya and the rest of Africa are still evolving neutrally. However, Omuut *et al.*, (2023) reported that populations in America and Asia are expanding.

The specimens collected from different regions in Kenya revealed the two types of strains are present in the FAW populations. This has been represented in the phylogenetic tree analysis where out of 49 sequences from different regions, 10 clustered with the C-strain and 39 with R-strain fall armyworm. Nagoshi *et al.*, (2018) also observed similar types of strains (corn and rice strains) from Africa (Togo) based on the COI gene. From this study, both the rice and corn strains collected from the eastern and central regions in Kenya actually clustered with the ones found in Northern hemisphere (India and USA), southern hemisphere (Northeast Brazil) and Eastern hemisphere (China: Gen Bank MT779914, MT779815, MT779838).

Haplotypes of 'C' strain collected from this study clustered with the Gen Bank sequences isolated from C strains from united states (GenBank: AY714300, AY714298, AY714299), North east Brazil (GenBank: KF624877 and KF624876) and India (GenBank: MK295625). R strain sequences from China clustered with 39 sequences from this study. This suggests that the invasion into Kenya could have originated from these regions.

Haplotype diversity of fall armyworm from regions in Kenya

In total, 19 haplotypes of *S. frugiperda* were detected from the 48 COI gene sequences isolated from specimens collected from eastern and central regions of Kenya. Among the 19 haplotypes,

most of samples collected (17) from these regions belonged to the 19th haplotype with samples from Kiambu having the highest number of samples in this haplotype. Haplotypes -1, hap-2, hap – 3 and hap – 4 belonged to the samples which had grouped together with the GenBank C-strains which had originated from either Northern hemisphere (USA and India) and southern hemisphere (N.E Brazil). The rest of the 14 haplotypes had grouped with the R strain. In this study, we checked distribution of the haplotypes in the counties and related them to the GenBank sequencies based on the grouping in the phylogenetic tree. Haplotypes one to haplotype five were associated with the GenBank sequences of known haplotypes isolated from Northern hemisphere (specifically central U.S.A and India) and the southern Hemisphere (N.E Brazil) while haplotypes six to 19 were similar to the ones from eastern Hemisphere especially China.

From the haplotype (Table 2), 39 COI gene sequences from Kenya (hap-6 to 19) were identical to natives from China (Eastern hemisphere) while 8 sequences from Kenya (hap 1 to 5) were identical to the natives from USA (Lewter *et al.,* 2006) and Brazil (Mastrangelo *et al.,* 2014). It means that fall armyworm from USA, Brazil, China and India have been involved in intercontinental dispersal specifically to countries in Africa including Kenya. The most parsimonious explanation for the introduction and invasion of fall armyworm into Africa and eventually to Kenya is a single introduction of either the eggs, larvae or adults followed by natural dispersion or conveyance within trade items.

Genetic differentiation of populations

F-statistics average over all the loci indicated fixation index of relatively high allelic homogeneity at a low Fst value of 0.10917 as shown in table 4. This indicated a low genetic differentiation within the populations. Fst measures the amount of genetic variance that can be explained by population structure based on Wrights F-statistics (Write, 1965). An Fst Value of 0 means no differentiation between the subpopulations while a value of 1 indicates complete differentiation (Luo *et al.,* 2019). This was further confirmed by the AMOVA results where the within populations (intragroup) variation accounted for 89.08% while among populations variation accounted for only 10.92% (Table 3). This suggests a high genetic exchange (gene flow) which might have led to low genetic differentiation between the populations (inter group) as suggested by the low nucleotide diversity Pi. Table 1 (C strain populations, 0.09279; R strain populations, 0.06695).

Data generated from this study indicated the Kenyan populations of *Spodoptera frugiperda* are more genetically diversified compared to the Asian ones. This was also observed by Acharya *et al*, (2021) as they compared African FAW to that of Asia. Within 'R' strain (n = 169) and 'C' strain (n = 14) populations, the nucleotide diversity was higher in 'C' strain than 'R' strain (Table 1). Fu's Fu test statistics was significantly negative (-0.31872), suggesting that the FAW population in Kenya might be undergoing expansion (Table 4). Mismatch distribution analysis for testing demographic expansion presented a graph with bimodal distribution (Figure 6). This was consistent with allopatric divergence followed by population growth (Eweleit *et al.,* 2015). Tajima's D test statistics was positive (0.11287) (Fig.4) suggesting the availability of two distinct types of population that are distinct from each other but they are favoured by the geographic region.

Host Diversity

The survey found only two alternative host plants species of *Spodoptera frugiperda* on the sampled regions; maize, napiergrass and sorghum, which are C4 plants belonging to the family of Poaceae (grass family). This is by far too few compared to 28 alternative hosts reported by Herlinda *et al*, (2022) in South Sumatra, 180 reported by Casmuz *et al*, (2010), 353 plants belonging to 76 different families reported by Montezano *et al*, (2018) in America and 29 alternative hosts reported by Jeannette *et al*, (2022) in southern and central Benin. The two hosts reported in this study were not new hosts as they had been reported by both Herlinda *et al.,* (2022) and Montezano *et al.,* (2018). The limited number can be attributed to fact that this study was only limited to one and half year period, in Kenya. Another reason could be the limited number of selected regions the study was carried on. This number is expected to increase as studies by Fiteni *et al.,* (2022) have proved that *Spodoptera frugiperda* has the ability to adapt to new host plants in due course of time, expanding its food sources as its population multiplies.

Ali *et al.,* (2023) observed that fall armyworm prefers the C4 plants over C3 plants, probably due to nutritional quality of C4 plants. This study is a follow up on the presence of fall armyworm on different crops which shows some effort in monitoring the establishment of and assists in management of fall armyworm in Kenya. The fact that a female *Spodoptera frugiperda* moth can lay eggs on host plants, non-host plants and even non plant materials show its potential to expand its hosts (Rojas *et al.,* 2018). It is also important to note that the best stage of development to study the impact of fall armyworm is the destructive larval stage other than the moth. The moth is difficult to localize as it keeps on moving. The polyphagous nature of fall armyworm can allow it to maintain its population outside the main cropping season. This may also bring pressure to force some to migrate. For effective pest management, it's important to know which plants are potential host for this pest.

Summary and Conclusions

This study confirmed the existence of *Spodoptera frugiperda* pest in Kenya in two strains- corn and rice strain. The pest was found to be a serious pest in maize and in one region, sorghum was also threatened. It was also confirmed that Rice strain was more in population (77.8 %) than Corn strain (22.2%) within the study region. These findings are also in conformity with Bhavani *et al.,* 2019 who reported prevalence of R strain over corn strain in India on sugarcane. Although C strain has been shown to occur majorly on maize (Padhee *et al.,* 2019), this study shows that R-strain has colonized maize in the studied regions in Kenya than C strain. Throughout the study fall armyworm was noted to be hosted mainly by maize and thus there is need to find out if it may shift to new hosts in absence of maize and maintain its population throughout the year as observed by Nagoshi and Meagher in 2004 in America. This study found that the two strains of fall armyworm were in a single host plant which means it is possible that the two strains were present in the propagule that introduced the pest in Africa. Similar findings were previously reported by Juárez *et al.,* 2014 who reported that the two strains coexist in sympatry in at least part of their distribution range.

Recommendations

Further studies should be done to conclusively establish how FAW reached Kenya and whether there is continuous introduction.

The establishment of permanent populations of fall armyworm and pattern of regional migrations needs to be determined.

Studies by Rodney *et al.,* 2021 indicate that the fall armyworm R-strain is not (yet) present

in significant numbers in Africa but this study indicated more R strain than C strain present in Kenya. Further studies need to be done to bring out clarity.

The larvae of *Spodoptera frugiperda* can feed on different crops as it develops (Paredes *et al.*, 2021) and therefore continuous studies should be done continuously to determine whether the pest has expanded its host plant range.

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