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Computational Approaches to Understanding Mosquito Insecticide Resistance: Genomic Insights into Esterase Dynamics

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Abstract: In this article, the molecular details of mosquito pesticide resistance are examined, with a focus on the function of detoxification genes, namely the carboxyl-esterase gene family. The study investigates resistance mechanisms, highlighting gene duplication, amplification, and genetic alterations. It discusses the evolution of monitoring techniques from traditional bioassays to molecular methods like microarrays and PCR. The three-dimensional organization of the enzyme superfamily is examined; emphasizing the alpha/beta hydrolase fold protein. The study presents a comparative nucleotide sequence analysis of mosquito esterase genes, revealing divergence among species. Nucleotide composition analysis indicates biases, and amino acid composition analysis identifies crucial residues. Exon-intron analysis uncovers variations in splice sites, suggesting functional constraints. Multiple sequence alignment and phylogenetic analysis provide insights into evolutionary relationships. In conclusion, the article discusses the significance of esterase genes in disease control amid increasing insecticide resistance. Future research priorities include structural investigations and functional characterizations, promising advancements in insect biochemistry.

Index Terms – Anopheles, Aedes, Culex, Insecticide resistance mechanisms, Detoxification genes, Phylogenetic analysis, Functional conservation, Computational genomics, Vector-borne diseases

I. INTRODUCTION

Insecticides will continue to be an essential part of managing mosquito carriers as long as this trait exists. On the other hand, the constant use of a restricted quantity of pesticides to control agricultural pests as well as animal and human illnesses has resulted in resistance, rendering the use of pesticides useless and reducing the range of options for disease management [1]. Since vector-borne infections are one of the main causes of morbidity and death, particularly in tropical and subtropical areas, insecticide-assisted vector control is essential to the management and prevention of infectious diseases. A key function for detoxification genes is played in the process of pesticide resistance. These are the genes that confer resistance to the harmful effects of hazardous chemicals on the body. Resistance to environmental toxins is provided by these genes. Humans and other species depend on these genes for survival. An investigation of the genome of *An. sinensis* identified 174 genes associated with detoxification, including 50 CCEs (choline/carboxyl-esterases), 31 GSTs, and 93 P450s [2].

1.1 Insecticide resistance-related enzyme system

Should chemical controls remain a component of integrated pest management techniques, then insecticide resistance monitoring programmes will become increasingly important. It is crucial to comprehend the molecular underpinnings of vector control techniques and pesticide resistance because of this. There are several ways to identify insecticide resistance, and bioassays and biochemical detection techniques have been used in the past to study it [3, 4, 5 and 6]. Molecular techniques like microarray approaches and mutation analysis based on polymerase chain reaction (PCR) are rapidly replacing these widely used methodologies [7, 8 and 9].

There are three mechanisms in which insecticide resistance resulting from metabolic detoxification might emerge in wild populations: gene duplication, gene amplification, and genetic alterations in coding areas [10]. Hemiptera and Diptera have both been shown to exhibit resistance brought on by esterase gene amplification, which is the mechanism behind the increased breakdown and/or sequestration of pyrethroids, carbamates, and OPs. In resistant species, there are many copies of the esterase gene, whereas in susceptible organisms it is generally found in a single copy [9, 11, 12, 13 and 14]. There has been evidence of different expression of genes in the orders Diptera, Lepidoptera, Hymenoptera, and Hemiptera, where resistant species are able to create more gene products than susceptible organisms [9, 13]. Lastly, it has been shown that the Diptera, Hymenoptera, and Lepidoptera contain mutations in the carboxyl esterase gene-encoding domains. In some situations, these mutations result in enhanced OP hydrolysis and/or sequestration [8, 13].

By shedding light on the intricate relationships across insect detoxification enzyme families, genomic and phylogenetic research is contributing to our knowledge of the genetic underpinnings of metabolic resistance to pesticides. Although additional esterases with

distinct domains could also be involved, most of the esterases involved in pesticide metabolism seem to belong to the carboxyl esterase gene family. Although early esterase studies concentrated on both naphthyl acetate substrates (alpha and beta), it is still uncertain how many of these isozymes actually belong to the carboxyl-esterase gene family. Furthermore, utilizing both naphthyl acetate and para-nitro phenyl acetate as a foundation for systematically evaluating the pesticide resistance of *Aedes aegypti* populations in Brazil has resulted in some conflicting findings. According to these findings, using biochemical tests to search for populations resistant to pesticides in the field requires the use of more recent and focused substrates [6].

There are few orthologues throughout insect species, and the family of carboxyl esterase genes seems to be expanding swiftly. Every type of insect has an own collection of genes that aid in detoxification. This family includes highly specialized enzymes with specific substrates as well as less selective members with broad and occasionally overlapping ranges of substrates, as previously mentioned [15, 16, 17 and 18]. It has long been thought that protein families, such as carboxyl esterases, that are commonly associated with drug resistance or degradation, develop rapidly. These proteins have demonstrated the ability to acquire many mutations without impairing their original function. They seem to have a selectivity advantage even if this procedure makes them less selective. The intricate processes involved in metabolic resistance to pesticides have been proven by microarray tests, and different resistant populations have evolved specific methods to cope with the poisons found in their environments. Undoubtedly, in insects, detoxifying gene families are growing quickly and species-specifically, indicating significant rates of diversification, due to gene redundancy may account for the variations seen in populations [18, 19, 20 and 21].

1.2 Enzyme superfamily and three-dimensional organization

There are A–C clades in the detoxifying group. Dipterans only had clade B present. The most abundant radiation among dipteran-specific α-esterase was nonetheless distinct from other insect orders. Since all esterase's belong to the fold protein alpha/beta hydrolase (Pfam PF00561 domain) gene family, which includes the Pfam PF00135 domain carboxyl esterase (or carboxyl/cholinesterase) family of genes [22, 23 and 24]. Numerous functionally distinct enzymes with a broad range of substrate hydrolyzability (i.e., substrates with varied chemical properties) have the hydrolase fold domain alpha/beta. For example, this superfamily includes, among other proteins, lipases, proteases, dehalogenases, peroxidases, esterases and epoxide hydrolases. It is one of the most common protein folds found in nature [23, 25 and 26].

Every carboxyl-esterase enzyme is composed of an alpha/beta-sheet, not a barrel, with eight strands connected by circumferential helices. The main DNA sequences of the proteins in this family range greatly in terms of their substrate specificity and do not share many similarities. As a result of their shared ancestor's structure and the residues' preserved arrangement in the catalytic region, esterases are nevertheless believed to have existed [26, 27]. The fact that these enzymes play so many different roles explains the rise in interest. Esterases are widely distributed and crucial for the metabolism of a variety of endogenous and foreign chemical groups. They carry out several vital tasks in the growth and behavior of insects, including the breakdown of odorants and activities pertaining to digestion and reproduction. Most of the esterases in insects are members of the carboxyl-esterase gene family hydrolyze a variety of essential compounds, including pheromones and other semiochemicals.

A global standard for the categorization of esterases has not yet been established, despite all the efforts to create a better system. In an effort to provide a unified approach for categorising esterases, the examples above encompass a variety of factors (such as substrate choice, the catalytic site and the protein's basic structure, in addition to molecular characteristics such nucleotide homology). The fact that different researches employ distinct categorization criteria for the same enzymes suggests that these approaches are not, however, facilitating the creation of a global standard. When examining pesticide resistance, for instance, most researchers follow the nomenclature established for the *Culex* mosquito genus based on the use of both naphthyl acetates as the base. Classifications based on these substrates, despite their low cost and ease of use, have been demonstrated to provide contradictory findings of pesticide resistance in both wild and cultured populations, and they fail to differentiate between the wide ranges of esterase isoforms found in insects. To discriminate between various metabolic resistance mechanisms, it is crucial to employ new and better techniques.

Currently, the phylogenetic criterion seems to be the most effective measure for categorising esterases, especially considering the increasing usage of sequencing techniques. Working together on microarray, biochemical, and genomic studies appears to be the best way to create a common understanding and classification of this complex gene family. Esterases are a class of rapidly changing proteins that play a critical role in the life cycle of mosquitoes. As such, they make an excellent molecular marker for phylogenetic connections and their classification among insects, and particularly among mosquitoes. These are excellent genes and gene products as models for examining their evolutionary history, according to a number of previously described areas of study. Thus, in order to investigate the evolution and functional conservation of mosquito esterase genes, a comparative study of nucleotide sequences has been conducted in this work.

II. METHODOLOGY

The esterase gene sequence database of many mosquito species viz. *Anopheles*, *Aedes* and *Culex* has been prepared following the similar methodology as described earlier [28-31]. The current study includes 60 genomic sequences belonging to three genuses (Table 1). Clustal W was used to carry out sequence alignments in order to identify conserved sequences. [32]. Esterase gene intron sequences were examined for the existence of conserved sequence motifs. The Genomics% GC content calculator was used to determine the composition of nucleotides (AT & GC concentration). To illustrate the gene's bias, a comparison study of the A+T and G+C contents was carried out. The UPGMA technique was also used for the phylogenetic analysis.

Table 1: List of the esterase gene genomic sequences from several mosquito species.

Sr. No	Organism	Species	Accession No.	Length (b.p.)	No. of exons	No. of introns	No. of amino acids (aa)
1.	*Anopheles*	*An. scanloni*	GU065046	462	-	-	153
2.		*An. baimaii*	GU065000	462	-	-	153
3.		*An. baimaii*	GU064973	462	-	-	153
4.		*An. baimaii*	GU064961	462	-	-	153
5.		*An. dirus*	GU065020	462	-	-	153
6.		*An. dirus*	GU065006	462	-	-	153
7.		*An. gambiae*	KP165384	2031	4	3	422
8.		*An. gambiae*	KP165369	2031	4	3	422
9.		*An. gambiae*	KP165335	2031	4	3	422
10.		*An. funestus*	DQ534435	1393	3	2	301
11.		*An. funestus*	JN815138	556	2	1	134
12.	*Aedes*	*Ae. albopictus*	HQ676572	653	-	-	198
13.		*Ae.albopictus*	AB218421	2182	-	-	702
14.		*Ae. aegypti*	EF209048	2721	-	-	702
13.	Culex	*Cx. quinquefasciatus*	GQ202028	1550	3	2	540
14.		*Cx. quinquefasciatus*	JQ341054	1165	2	1	476
15.		*Cx. quinquefasciatus*	JQ341053	936	2	1	56
16.		*Cx. pipiens*	EF471908	861	7	6	56
17.		*Cx. pipiens*	EF471907	860	2	1	266
18.		*Cx. pipiens*	EF471906	860	2	1	260
19.		*Cx. pipiens*	HQ881780	568	3	2	146
20.		*Cx. pipiens*	HQ881779	568	3	2	146
21.		*Cx. pipiens*	HQ881778	568	3	2	146
22.		*Cx. pipiens*	HQ881775	568	3	2	146
23.		*Cx. pipiens*	HQ881775	568	3	2	146
24.		*Cx. pipiens*	HQ881762	566	3	2	146
25.		*Cx. pipiens*	HQ881761	566	3	2	146
26.		*Cx. pipiens*	HQ881760	568	3	2	146
27.		*Cx. pipiens*	HQ881756	555	3	2	146
28.		*Cx. pipiens*	HQ881753	568	3	2	146
29.		*Cx. pipiens*	HQ540609	268	-	-	88
30.		*Cx. pipiens*	HQ540608	268	-	-	88
31.		*Cx. pipiens*	HQ540607	268	-	-	88
32.		*Cx. pipiens*	AM949567	1815	7	6	490
33.		*Cx. pipiens*	AM773727	1087	2	1	341
34.		*Cx. pipiens*	EF614456	297	-	-	95
35.		*Cx. pipiens*	EF614450.1	297	-	-	95
36.		*Cx. theileri*	HQ893667	287	2	1	74
37.		*Cx. pipiens*	JQ780068.1	714	3	2	199
38.		*Cx. pipiens*	JQ812615	942	2	1	56
39.		*Cx. pipiens*	JQ812614	713	3	2	199
40.		*Cx.pipiens*	KC687140	1980	7	6	266
41.		*Cx.pipiens*	KC687138	1968	7	6	540
42.		*Cx.pipiens*	KC687136	1972	7	6	540
43.		*Cx.pipiens*	KC687141	2914	4	3	540
44.		*Cx.pipiens*	KC687139	2864	4	3	540
45.		*Cx.pipiens*	AY545984	2910	4	3	540
46.		*Cx.pipiens*	AY545983	1984	7	6	540
47.		*Cx.pipiens*	JQ866911	2506	4	3	540
48.		*Cx.pipiens*	JQ866910	2864	4	3	540
49.		*Cx.pipiens*	JQ866909	1967	7	6	533
50.		*Cx.pipiens*	M32328	3105	4	3	540
51.		*Cx.pipiens pallens*	AY762905	2085	-	-	694
52.		*Cx. quinquefasciatus*	EF174327	1997	4	3	540
53.		*Cx. quinquefasciatus*	AFJ12120.1	713	3	2	199
54.		*Cx.tritaeniorhynchus*	AB122152	3439	-	-	701
55.		*Cx.tritaeniorhynchus*	AF177382	6028	4	3	540
56.		*Cx.nigripalpus*	KM190929	1808	-	-	581
57.		*Cx. sitiens*	EU710675	389	2	1	71
58.		*Cx. palpalis*	EU710673	382	2	1	71

59.		*Cx. annulirostris*	EF710628	382	2	1	71
60.		*Cx. annulirostris*	EU710628	382	2	1	71

III. RESULTS AND DISCUSSION

Three different mosquito species' esterase genes having entire and partial nucleotide sequences (60) that may be found in a number of NCBI, GenBank, and other database sites (Table 1) has been examined using a range of bioinformatic techniques.

3.1 Nucleotide Similarity Analysis

The lengths of the esterase genes may be linked together, despite their significant divergence from one another. Despite the varied number and locations of exons in each reported sequence, it was discovered that the nucleotide coding sequences were often identical. Furthermore, there are significant sequence similarities found in the esterase genes of mosquitoes (Table 1).

3.2 Nucleotide Base Composition Analysis

The whole esterase coding region's nucleotide makeup was examined. The GC content of *Culex* mosquitoes was greater than that of all the data studied (Table 2). With the exception of the third codon position, the A content at the first and second codon positions was found to be marginally greater than the T content, indicating bias in the nucleotide composition. The mosquito esterase genes' predilection is evidently shown by a high AT content. Since no similar study appears to have been done previously and more research is required for justification, the results seen could not be compared.

3.3 Amino Acid Composition Analysis

Table 3 displays the results of the analysis of the esterase gene's amino acid composition. Three amino acid residues—valine, serine, and leucine—which make up around 20–30% of all the amino acids in all the mosquito species examined were found to be highly concentrated in the protein. This finding suggests that these residues may be biologically highly demanded throughout development. This extraordinary conduct requires more in-depth investigation.

3.4 Exon-Intron Analysis

The esterase genes of only *Culex* mosquitoes were found to have a maximum of six introns (Tables 4-5). The Introns of these genes did not exhibit any discernible sequence conservation. On contrary, Table 4 revealed a noteworthy conservation trend for the intron-exon splice locations. The conserved splice sites may suggest that there were functional limitations during evolution that were necessary to preserve the genes' overall functionality.

Table 1: Percent identity of Esterase genes among *Aedes* and *Culex* mosquitos

Sr. No.	Organism	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	*Ae. aegypti*	100	91	-	-	-	-	-	-	-	-	-	-	-	-	-	-	80	79
2	*Ae. aegypti*	91	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	80	79
3	*Cx. pipiens*	-	-	100	94	94	-	-	-	99	93	-	-	94	-	-	87	-	-
4	*Cx. pipiens*	-	-	94	100	94	-	-	-	94	93	-	-	99	-	-	-	-	-
5	*Cx. pipiens*	-	-	94	94	100	-	-	-	93	92	-	-	94	-	-	89	-	-
6	*Cx. pipiens*	-	-	-	-	-	100	96	99	-	-	93	96	-	94	81.0	-	-	-
7	*Cx. pipiens*	-	-	-	-	-	96	100	95	-	-	92	100	-	96	82	-	-	-
8	*Cx. pipiens*	-	-	-	-	-	99	95	100	-	-	93	95	-	94	81.0	-	-	-
9	*Cx. pipiens*	-	-	99	94	93	-	-	-	100	93	-	-	94	-	-	89	-	-
10	*Cx. pipiens*	-	-	93	93	92	-	-	-	93	100	-	-	93	-	-	82	-	-
11	*Cx. pipiens*	100	-	80	-	-	93	92	93	-	-	100	92	-	90	81.0	-	-	-
12	*Cx. pipiens*	100	67	78	97	-	96	100	95	-	-	92	100	-	96	82	-	-	-
13	*Cx. pipiens*	-	-	94	99	94	-	-	-	94	93	-	-	100	-	-	-	-	-
14	*Cx. pipiens*	-	-	-	-	-	94	96	94	-	-	90	96	-	100	81	-	-	-
15	*Cx. tritaeniorhynchus*	-	-	-	-	-	81	82	81	-	-	81	82	-	81	100	-	-	-
16	*Cx. nigripalpus*	-	-	-	-	89	-	-	-	89	82	-	-	-	-	-	100	-	-
17	*Cx. pipiens*	80	80	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	-
18	*Cx. tritaeniorhynchus*	79	79	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100

Table 2: Nucleotide Base Composition Analysis

Sr. No.	Organism	Accession No.	Length (b.p.)	A	T	G	C	% GC	% AT
1.	*Ae. aegypti*	EF209048	2721	759	662	656	644	47.8	52.2
2.	*Ae. aegypti*	AB218421	2182	570	510	557	545	50.5	49.5
3.	*Cx. pipiens*	KC687140	1980	508	504	551	417	48.9	51.1
4.	*Cx. pipiens*	KC687138	1968	494	511	547	416	48.9	51.1
5.	*Cx. pipiens*	KC687136	1972	504	503	548	417	48.9	51.1
6.	*Cx. pipiens*	KC687141	2914	841	769	684	620	44.7	55.3
7.	*Cx. pipiens*	KC687139	2864	820	756	667	621	45	55
8.	*Cx. pipiens*	AY545984	2910	838	768	633	621	44.8	55.2
9.	*Cx. pipiens*	AY545983	1984	508	503	550	423	49	51
10.	*Cx. pipiens*	EF174327	1997	505	517	550	425	48.8	51.2
11.	*Cx. pipiens*	JQ866911	2506	706	649	606	545	45.9	54.1
12.	*Cx. pipiens*	JQ866910	2864	820	756	667	621	45	55
13.	*Cx. pipiens*	JQ866909	1967	493	511	547	416	49	51
14.	*Cx. pipiens*	M32328	3105	904	830	700	671	44.2	55.8
15.	*Cx. tritaeniorhynchus*	AF177382	6028	1925	1821	1136	1146	37.9	62.1
16.	*Cx. nigripalpus*	KM190929	1807	458	438	512	399	50.4	49.6
17.	*Cx. pipiens*	AY762905	2805	467	415	600	603	57.7	42.3
18.	*Cx. tritaeniorhynchus*	AB122152	3439	901	765	851	922	51.6	48.4

Table 3: Amino Acid Composition Analysis

Sr No	Organism	Accession No.	Amino acid (a.a)	Ala (A)	Arg (R)	Asn (N)	Asp (D)	Cys (C)	Gln (Q)	Glu (E)	Gly (G)	His (H)	Ile (I)	Leu (L)	Lys (K)	Met (M)	Phe (F)	Pro (P)	Ser (S)	Thr (T)	Trp (W)	TyR (Y)	Val (v)	Pyl (O)	Sec (U)	Mol.wt.	PI
1	*Ae. aegypti*	EF209048	702	6.1	6.3	4.8	5.1	1.7	2.4	5.8	7.4	3	3.7	10.4	3.3	1.1	4.1	6.8	8.1	7	1.9	3.7	7.1	0.0	0.0	78193.3	6.11
2	*Ae. aegypti*	AB218421	702	6.7	6.4	5	5	1.6	2.4	6	7.3	3	3.7	10.5	3.3	1.1	4.1	6.6	8.1	6.7	1.9	3.7	7	0.0	0.0	78219.3	6.18
3	*Cx. pipiens*	KC687140	266	3.7	4.8	6.9	5.2	1.7	3.5	6.9	8.1	1.9	5.2	9.3	5.7	3.0	5.6	5.7	6.9	3.9	1.3	4.3	6.7	0.0	0.0	61261.7	5.80
4	*Cx. pipiens*	KC687138	540	4.1	4.8	6.7	5.4	1.7	3.3	6.7	8.1	1.9	4.8	9.3	5.7	3.0	5.6	5.7	6.9	3.7	1.3	4.3	7.2	0.0	0.0	61117.6	5.80
5	*Cx. pipiens*	KC687136	540	4.1	4.8	6.3	5.2	1.7	3.5	6.5	8.1	1.9	5.2	9.6	6.1	3.0	5.6	5.7	7.0	3.7	1.3	4.1	6.7	0.0	0.0	61108.8	6.26
6	*Cx. pipiens*	KC687141	540	6.9	5.2	3.9	5.9	1.9	4.8	5.9	7.8	2.0	5.0	9.4	5.2	3.0	5.2	5.7	5.9	5.0	1.5	3.9	5.9	0.0	0.0	60750.4	5.85
7	*Cx. pipiens*	KC687139	540	7.0	5.2	3.7	5.9	1.9	4.8	5.9	8.0	2.0	5.6	9.3	5.2	2.8	5.4	5.7	5.6	5.0	1.5	3.9	5.7	0.0	0.0	60733.4	5.85
8	*Cx. pipiens*	AY545984	540	6.9	5.2	3.9	5.9	1.9	4.8	5.9	7.8	2.0	5.0	9.4	5.0	3.0	5.2	5.7	5.9	5.2	1.5	3.9	5.9	0.0	0.0	60723.3	5.75
9	*Cx. pipiens*	AY545983	540	3.7	5.0	6.9	5.2	1.7	3.5	6.9	8.1	1.9	5.2	9.4	5.7	3.0	5.4	5.7	6.9	3.9	1.3	4.3	6.5	0.0	0.0	61284.8	5.90
10	*Cx. pipiens*	EF174327	540	3.9	4.8	6.5	5.2	1.7	3.1	6.7	8.1	1.9	5.2	9.4	5.9	3.0	5.6	5.9	6.9	3.7	1.3	4.3	7.0	0.0	0.0	61154.8	6.01
11	*Cx. pipiens*	JQ866911	540	6.3	5.2	4.1	5.9	1.9	4.8	5.7	7.6	1.9	5.0	9.8	5.4	3.0	5.2	5.7	6.1	5.0	1.7	3.9	5.9	0.0	0.0	60955.7	6.00
12	*Cx. pipiens*	JQ866910	540	7.0	5.2	3.7	5.9	1.9	4.8	5.9	8.0	2.0	5.6	9.3	5.2	2.8	5.4	5.7	5.6	5.0	1.5	3.9	5.7	0.0	0.0	60733.4	5.85
13	*Cx. pipiens*	JQ866909	533	4.1	4.7	6.8	5.4	1.7	3.4	6.6	8.1	1.9	4.9	9.2	5.6	3.0	5.4	5.8	6.9	3.8	1.3	4.3	7.1	0.0	0.0	60287.6	5.70
14	*Cx. pipiens*	M32328	540	6.9	5.6	3.7	5.9	1.9	4.6	5.7	8	2.2	5.4	9.4	5.2	2.8	5.2	5.7	5.6	5.0	1.5	3.9	5.9	0.0	0.0	60806.5	6.21
15	*Cx. tritaeniorhynchus*	AF177382	540	6.1	4.4	4.4	6.5	2.0	4.8	5.6	7.8	2.0	4.3	8.9	5.6	3.3	5.4	6.5	5.7	5.0	1.5	3.9	6.3	0.0	0.0	60747.2	5.57
16	*Cx. nigripalpus*	KM190929	581	4.0	5.3	6.5	4.6	1.9	2.8	6.9	8.3	2.1	5.9	8.6	6.0	2.8	5.7	5.7	6.2	4.1	1.2	4.0	7.4	0.0	0.0	65815.6	6.87
17	*Cx. pipiens*	AY762905	694	6.8	6.9	4.6	5.0	1.6	2.6	5.9	7.2	2.9	3.7	10.7	3.2	1.2	3.9	6.2	8.5	7.1	1.9	3.3	6.9	0.0	0.0	77224.2	6.39
18	*Cx. tritaeniorhynchus*	AB122152	701	7.3	7.0	4.9	4.6	1.6	2.7	6.3	7.1	2.9	3.9	10.7	3.1	1.3	3.9	6.4	8.7	6.3	1.9	3.3	6.4	0.0	0.0	77986.1	6.49

Table 4: Analysis of Intron boundaries of *Culex* Esterase genes

Sr No	Species	No. of Exons	No. of Introns	Intron 1 (5'-3')	Intron 2(5'-3')	Intron 3(5'-3')	Intron 4(5'-3')	Intron 5(5'-3')	Intron 6 (5'-3')
1	*Cx. pipiens*	7	6	G/GTAGG -TTTAG/G	C/GTAAG-TGTAG/C	G/GTATG-TTCAG/G	T/GTAAG-TGTAG/G	G/GTAAG-CACAG/C	G/GTGAG-TACAG/G
2	*Cx. pipiens*	7	6	G/GTAGG-TTCAG/G	C/GTAAG-TGTAG/C	G/GTATG-TTCAG/G	T/GTAAG-TGTAG/G	G/GTAAG-CACAG/C	G/GTGAG-TACAG/G
3	*Cx. pipiens*	7	6	G/GTAGG-TCTAG/G	C/GTAAG-TGTAG/C	G/GTATG-TTCAG/G	T/GTAAG-TGTAG/G	G/GTAAG-CACAG/C	G/GTAAG-TACAG/G
4	*Cx. pipiens*	4	3	G/GTGAG-TTCAG/G	G/GTGAG-TCCAG/A	T/GTAAG-TCCAG/A	-	-	-
5	*Cx. pipiens*	4	3	G/GTGAG-TTCAG/G	G/GTGAG-TCCAG/A	T/GTAAG-TCCAG/A	-	-	-
6	*Cx. pipiens*	4	3	G/GTGAG-TTCAG/G	G/GTGAG-TCCAG/A	T/GTAAG-TCCAG/A	-	-	-
7	*Cx. pipiens*	7	6	G/GTAGG-TTTAG/G	C/GTAAG-TGTAG/C	T/ATGAT-TCAGA/T	T/GTAAG-TGTAG/G	G/GTAAG-CACAG/C	G/GTGAG-TACAG/G
8	*Cx. pipiens*	7	6	G/GTAGG-TTTAG/G	C/GTAAG-GTGGA/C	G/GTATG-TTCAG/G	T/GTAAG-TGTAG/G	G/GTAAG-CACAG/C	G/GTGAG-TACAG/G
9	*Cx. pipiens*	4	3	G/GTGAG-TCCAG/G	G/GTGAG-TCCAG/A	T/GTAAG-TCCAG/A	-	-	-
10	*Cx. pipiens*	4	3	G/GTGAG-TTCAG/G	G/GTGAG-TCCAG/A	T/GTAAG-TCCAG/A	-	-	-
11	*Cx. pipiens*	7	6	C/GTTGG-TTCAG/G	C/GTAAG-TGTAG/C	G/GTATG-TTCAG/G	T/GTAAG-TGTAG/G	G/GTAAG-CACAG/C	G/GTGAG-TACAG/G
12	*Cx. pipiens*	4	3	G/GTGAG-TTCAG/G	G/GTGAG-TCCAG/A	T/GTAAG-TCCAG/A	-	-	-
13	*Cx. tritaeniorhynchus*	4	3	G/GTGAG-TTCAG/C	G/GTCAG-CTAAG/G	C/GTAAG-AGCGA/T	-	-	-

Table 5: Exon-Intron size analysis (bp) in *Culex* Esterase genes

Sr No	Species	Exon 1	Intron 1	Exon 2	Intron 2	Exon 3	Intron 3	Exon 4	Intron 4	Exon 5	Intron 5	Exon 6	Intron 6	Exon 7
1	*Cx. pipiens*	141	53	138	73	151	56	392	57	345	53	255	65	201
2	*Cx. pipiens*	141	53	138	70	151	56	392	57	345	53	255	55	201
3	*Cx. pipiens*	141	54	138	73	151	56	392	57	335	53	255	56	201
4	*Cx. pipiens*	141	1171	138	59	882	63	462	-	-	-	-	-	-
5	*Cx. pipiens*	141	1121	138	61	882	63	462	-	-	-	-	-	-
6	*Cx. pipiens*	141	1167	138	59	882	63	462	-	-	-	-	-	-
7	*Cx. pipiens*	141	67	138	75	153	56	390	58	345	59	255	55	201
8	*Cx. pipiens*	141	62	138	74	151	56	392	4	345	53	255	55	201
9	*Cx. pipiens*	141	766	138	56	882	61	462	-	-	-	-	-	-
10	*Cx. pipiens*	141	1119	138	61	882	61	462	-	-	-	-	-	-
11	*Cx. pipiens*	120	74	138	70	151	56	392	57	345	50	255.0	55	201
12	*Cx. pipiens*	141	774	138	52.0	882	61	462	-	-	-	-	-	-
13	*Cx. tritaeniorhynchus*	141	3489	138	306	882	66	462	-	-	-	-	-	-

3.5 Alignment of Multiple Sequences and Phylogenetic Analysis

The Esterase gene was subjected to multiple sequence alignment in *Aedes* and *Culex* mosquitoes. Significant conservation is seen throughout the whole gene according to the alignment. Interestigly, the initiation codon show significant positional conservation along with many stretches of nucleotide conservation in the gene. The evolutionary relationships of the organisms inside the mosquito were ascertained by examining the phylogram based on the Esterase gene of mosquitos individually. *Aedes* and *Culex's* phylogenetic trees are displayed in (Figure 1). The mosquitoes display many clade groupings. The tree grouped in subdued, systematic ways.

4. Discussion and Conclusion

A lot of work has gone into developing a strong plan to prevent illnesses spread by mosquitoes in recent years. The growing pesticide resistance has made it difficult to find effective therapies. Computational approaches are important for disease prevention because of their rapid and promising results. Considering the high sequence similarity rates and overall protein design conservation, it seems sense to consider critical approaches to simultaneously target the gene in several species. The esterase gene showed significant results from amino acid composition analysis indicating the requirement of certain amino acids importantly in embryonic development. Earlier also, certain genes of mosquito showed significant expression patterns after bold meal induction along with amino acid codon usage biasness indicating their significant evolutionary correlation [28, 33]. It is confirmed that genes are changing more quickly while maintaining the overall function through structural and sequential variations between individual introns. Sequence comparisons of the esterase genes revealed a large number of nonsynonymous substitutions outside of conserved areas, indicating that the genes are developing in tandem with specific functional constraints. Further evidence of the divergence comes from differences in the size and positioning of exons and introns. Additionally, this demonstrated the strong bias in mosquito esterase genes. The intricate terrain of vector control tactics is shown by the study of mosquito esterase genes and

their consequences for pesticide resistance. The study's findings on resistance mechanisms, including gene duplication and alterations, shed light on the adaptive strategies employed by mosquitoes in response to constant pesticide exposure. The evolving methods for monitoring resistance, transitioning from conventional bioassays to molecular techniques, highlight the need for more precise and comprehensive approaches in understanding and addressing resistance patterns. The three-dimensional organization of the enzyme superfamily, particularly the alpha/beta hydrolase fold protein, provides valuable insights into the structural foundations of esterase genes. This understanding is pivotal for unraveling their diverse roles and substrate hydrolyzability, contributing to the broader field of insect biochemistry. The study's comprehensive analysis of nucleotide sequences, amino acid compositions, and exon-intron patterns across different mosquito species adds depth to our understanding of the genetic and functional variations within the esterase gene family. The discussion extends to the practical implications of these findings in disease control strategies. The identification of conserved sequences and functional constraints within esterase genes opens avenues for targeted interventions. The study's emphasis on the high rates of divergence among mosquito esterase genes, coupled with their conserved functions, suggests a delicate balance between evolutionary adaptation and the preservation of essential biological roles. In conclusion, as insecticide resistance becomes an escalating challenge, the study underscores the urgency of developing novel and effective control measures. The promising outcomes of computational techniques in understanding esterase gene dynamics hint at future possibilities for precision-targeted interventions. The ongoing genomic initiatives and collaborative research efforts are expected to yield significant advancements, providing a foundation for innovative approaches in mosquito-borne disease management. Looking ahead to promising discoveries in the larger field of insect biochemistry and metabolism, the paper urges more investigation into the structural and functional features of carboxyl-esterase genes.

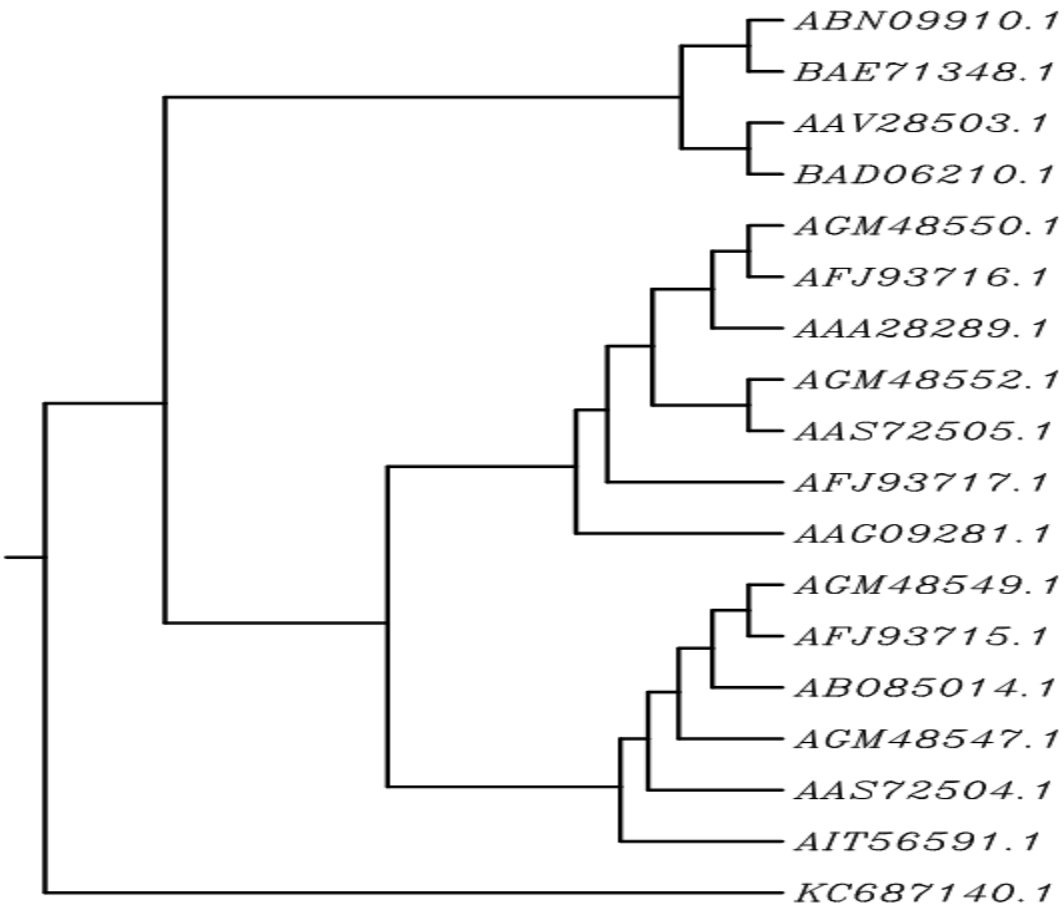

Figure 1: Phylogenetic tree of esterase genes belonging to *Aedes & Culex* mosquito by UPGMA Method

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