

Distribution of Voltage Gated Sodium Channel (VGSC) Gene Mutational Variation and Acetylcholinesterase-1 (ACE-1) as A Marker For Insecticide Resistance In Culex spp. Mosquitoes In Surabaya

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KEYWORDS	ABSTRACT
mosquitoes; culex spp; VGSC; ACE-1	Culex spp. is a vector for Japanese Encephalitis (JE) and filariasis in Indonesia. JE is caused by Japanese Encephalitis Virus (JEV). JEV attacks the central nervous system and causes inflammation of the brain, lifelong disability, and even death. Filariasis is a chronic infectious disease that can cause permanent disability. Transmission of JE and filariasis can increase due to the density of Culex spp mosquitoes. The purpose of this study was to identify the distribution of mutation variations in the Voltage Gated Sodium Channel (VGSC) and acetylcholinesterase-1 (ace-1) genes in Culex spp. mosquitoes in Surabaya. Mutation variations of the VGSC Culex spp. gene show that there are three genotypes, namely wildtype (LL), heterozygous (LS, FS), and homozygous (FF, SS). Heterozygous (FS) and homozygous (FF, FS) genotypes caused by point mutations in codon 1014 of the VGSC gene are found in the Culex spp. mosquito population in Surabaya. Heterozygous (LF) genotype was not detected in the Culex spp. mosquito population in Surabaya. Another study in Nigeria showed that homozygous/FF genotypes (45.1%) were more prevalent than heterozygotes/LF (36.3%) and wildtypes/LL (18.6%).

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1. Introduction

In recent decades it is known that the use of chemical insecticides is still often used for vector management related to infectious diseases. Excessive and poorly planned use leads to insecticide resistance in mosquito populations. This resistance results in insecticides no longer being effective and failing to control mosquito populations (Rai & Saha, 2022).

Molecular diagnosis is one step that can be taken to monitor the incidence of insecticide resistance. The basis for the diagnosis is the identification of genetic markers. Such identification is useful to increase knowledge and reduce the effects of insecticide resistance. Knockdown Resistance (KDR) is a mutation related to resistance to DDT and pyrethroids (Mwagira-Maina et al., 2021). The resistance mechanism of DDT and pyrethroids most commonly occurs due to L1014F Knockdown Resistance (KDR) mutations in voltage-gated sodium channel (VGSC) genes (Li et al., 2016). VGSC is a gene in the neurons of Culex spp. mosquitoes and plays a role in the regulation of sodium channels that have functions to control impulses and membrane excitability (Dong et al., 2014). In addition,

mutations can also occur in the acetylcholinesterase-1 (ace-1) gene. The ace-1 gene is located in the central nervous system of the mosquito *Culex* spp. Mutations result in gene insensitivity so that sensitivity decreases (Wang et al., 2022).

Culex is the most common vector in the spread of Japanese Encephalitis (JE) in Indonesia. JE is transmitted by the *Culex* spp. mosquito which is more active at night and sucks blood from animals and humans. The risk of JE transmission may increase due to the high density of *Culex* spp. mosquitoes and available reservoirs. This is especially true in areas with high agricultural and livestock culture (Kardena et al., 2021). *Culex* spp. mosquitoes are vectors of Lymphatic Filariasis (LF) and Japanese Encephalitis (JE). LF is an infectious disease that can be caused by the filarial worms *Wuchereria bancrofti* (90% of cases), *Brugia malayi*, or *Brugia timori*. *Culex* spp. mosquitoes become infected when biting and sucking host blood containing microfilariae. Microfilariae will undergo maturation into infective larvae inside mosquitoes. When an infected mosquito bites a person, the adult larva will deposit in the skin, migrate to lymphatic vessels, and develop into filarial worms (WHO, 2023). JE is an infectious disease caused by the Japanese Encephalitis Virus (JEV) and transmitted by the *Culex* mosquito species.

JEV transmission occurs through the bite of an infected mosquito. The JEV cycle circulates among mosquitoes, pigs, and/or waterbirds (enzootic cycle). Rural or suburban areas are where most cases are found because in these areas it is possible that humans will be closer to the JEV host. Japanese Encephalitis (JE) is an infectious disease caused by Japanese Encephalitis Virus (JEV). JEV comes from the genus *Flavivirus* with its mechanism of action is to attack the central nervous system. This results in inflammation of the brain, lifelong disability, to death in sufferers. JE is transmitted through mosquito vectors, especially *Culex tritaeniorhynchus*. Pigs and birds living in swamps are amplifying hosts. It was reported that JE cases in 2015 (40 people), 2016 (43 people), 2017 (6 people), 2018 (6 people), and 2019 (10 people). This data was obtained from JE surveillance in 11 provinces of Indonesia, namely Bali, West Kalimantan, East Nusa Tenggara (NTT), North Sulawesi, DI Yogyakarta, DKI Jakarta, Riau Islands, Central Java, Gorontalo, West Sulawesi, and Southeast Sulawesi. (Ministry of Health, 2020)

It is known that infectious diseases transmitted by mosquitoes are still a problem in Indonesia. Insecticides containing pyrethroids are often used to control adult mosquitoes, especially during outbreaks (Gao, Chen, Shi, Peng, & Ma, 2018). This can be attributed to the outbreak in Surabaya City which is one of the endemic areas of Dengue Virus Infection (DVI). Insecticides that are used continuously will affect not only *Aedes* mosquitoes. All mosquitoes including *Culex* will be exposed (Wila, Satoto, & Mujiyanto, 2020). This research is important to do because resistance data based on mutation markers of the Voltage Gated Sodium Channel (VGSC) and acetylcholinesterase-1 (ace-1) genes in *Culex* spp. mosquitoes in Surabaya are still rare or unknown.

The purpose of this study was to identify the distribution of mutation variations in the Voltage Gated Sodium Channel (VGSC) and acetylcholinesterase-1 (ace-1) genes in *Culex* spp. mosquitoes in Surabaya.

2. Materials and Methods

The type of research conducted is observational analytic with cross sectional as the research design. Adult *Culex* mosquito populations were targeted in the study. The population was obtained from several areas in the city of Surabaya, namely Sawahan, Kenjeran, and Tenggilis districts. The regions were selected based on areas with high (Sawahan), low (Kenjeran), and negative (Tenggilis) DVI cases transmitted by *Aedes* mosquitoes. *Culex* and *Aedes* mosquitoes in the same population do not compete with each other. *Culex* mosquitoes are more resistant to environmental exposure than *Aedes* so they are more numerous (Santana-Martínez, Molina, & Dussán, 2017) *Culex* mosquito breeding sites are mainly in places with relatively stagnant puddles such as sewers (Liu et al., 2019).

The samples in this study were adult *Culex* mosquitoes located in Sawahan (4 points), Kenjeran (4 points), and Tenggilis (2 points) sub-districts with a distance of 100 meters each. The

distance between the points was chosen based on the Aedes flight distance, which is 106 meters (Moore & Brown, 2022). The sample collection method is to use mosquito traps placed for 2 consecutive days in each sub-district for 6 months. The criteria for the selected sample were relatively homogeneous physiological conditions of mosquitoes and identification was carried out based on the characteristics of Culex mosquitoes.

The technique used in sampling is cluster random sampling. Researchers divide populations into groups or clusters. Sampling was carried out using SBT traps (see figure 1) in Surabaya City, precisely Sawahan, Kenjeran, and Tenggilis Districts using mosquito traps placed inside and outside the house for 2 consecutive days and changed every 12 hours. The traps needed in Sawahan (high IVD cases) and Kenjeran (low IVD cases) sub-districts are 8. Such traps are placed on four houses spaced 100 m each. The selected houses consist of two houses with patients who have been infected and two houses with patients who have never been infected with the Dengue virus. Meanwhile, in Tenggilis sub-district with negative IVD cases or as a control, 4 mosquito traps are needed. Placement of mosquito traps was carried out in two houses with patients who had never been infected with the Dengue virus.

The capture of adult stage Culex spp. mosquitoes was carried out in several areas of Surabaya City, namely Sawahan, Kenjeran, and Tenggilis Districts. Fishing is carried out with mosquito traps laid on the territory. The sample will be examined at the Malaria Laboratory which is part of the Institute of Tropical Diseases, Universitas Airlangga. This study was conducted from June to November 2022 or for 6 months.

3. Result and Discussion

Environmental Conditions of the Sampling Site

Tenggilis District

The sampling site is located inside the housing with a road width of 5 meters. The number of houses in a row is 18. The width of each house is about 9 meters. The surrounding conditions of the sampling environment are relatively clean. Piles of used items were not visible around the study site. The sewers around the location point are closed and the water flow is smooth.

Kenjeran District

The sampling site is located inside an alley with a width of 1.5 meters. The number of houses in a row is 42. The width of each house is about 3 meters. The surrounding conditions of the sampling environment are relatively dirty. Piles of used items were seen around the research site. The sewers around the location are closed and the water flow is smooth.

Sawahan District

The sampling site is located inside an alley with a width of 2 meters. The number of houses in a row is 71. The width of each house is about 5 meters. The surrounding conditions of the sampling environment are relatively dirty. Piles of used items were seen around the research site. The sewers around the location are open and the water flow is not smooth.

Distribution of Culex spp. mosquitoes.

Distribution of Culex spp. mosquitoes in Tenggilis sub-district

Total mosquitoes Culex spp. in Tenggilis sub-district it is 65. Culex mosquito spp. more active at night. Distribution of mosquitoes Culex spp. the highest in September 2022 and the lowest in January 2023. (see figure 1)

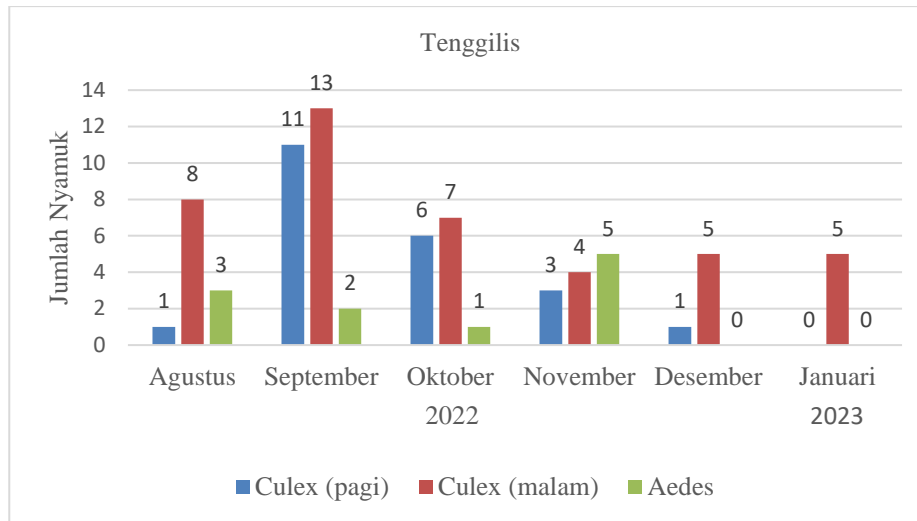


Figure 1 Distribution of Culex spp. Mosquitoes in Tenggilis District

Distribution of Culex spp. mosquitoes. in Kenjeran sub-district

Total mosquitoes Culex spp. in Kenjeran sub-district it is 101. Culex mosquito spp. more active at night except December 2022. Distribution of mosquitoes Culex spp. the highest in September 2022 and the lowest in January 2023. (see figure 2)

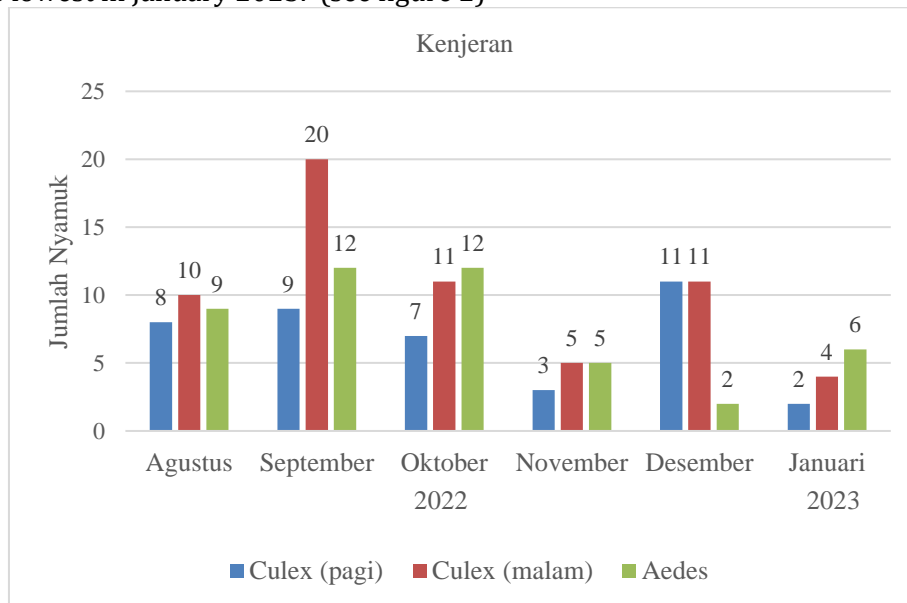


Figure 2 Distribution of Culex spp. mosquitoes. in Kenjeran District

Distribution of Culex spp. mosquitoes. in Sawahan sub-district

Total mosquitoes Culex spp. in Sawahan sub-district it is 111. Culex mosquito spp. more active at night except September 2022. Distribution of mosquitoes Culex spp. the highest in September 2022 and the lowest in November 2022. (see figure 3)

Moon	August	September	October	November	December	January
Temperature (°C)	23-28	24-29	23-27	24-27	24-28	26-28
Moisture (%)	70-100	70-85	80-100	85-95	80-100	85-95
Wind Speed (km/h)	10-20	10-20	10-20	0-20	10-20	10-20
Rainfall (mm)	7	9	27	69	171	281

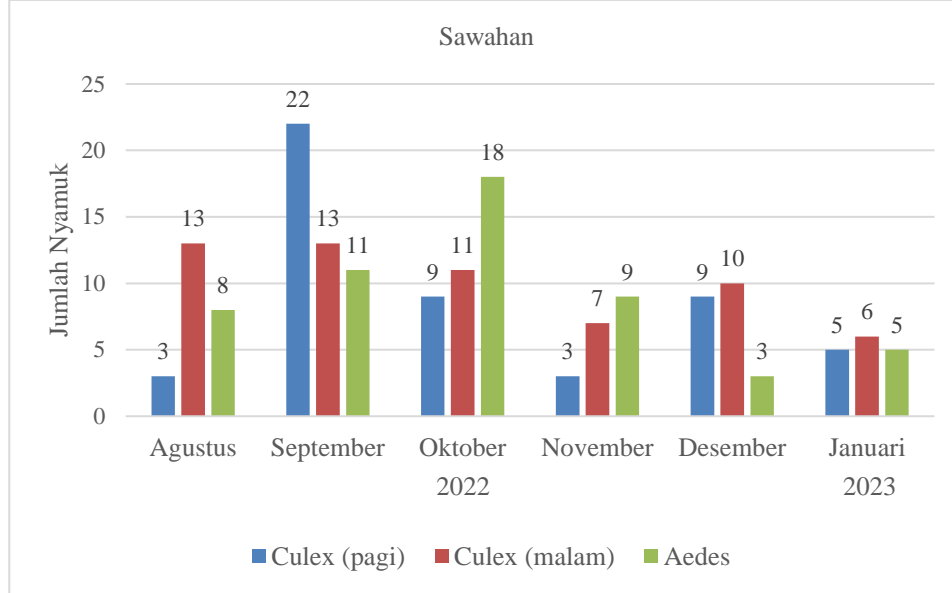


Figure 3 Distribution of Culex spp. mosquitoes. in Sawahan District

Distribution of Culex spp. mosquitoes. in Surabaya

Total mosquitoes Culex spp. in Surabaya it is 277. Culex mosquito spp. more active at night. Distribution of mosquitoes Culex spp. the highest in September 2022 and the lowest in January 2023. Distribution of mosquitoes Culex spp. Significantly different in both months even though the climatic conditions are relatively the same. (see figure 4)

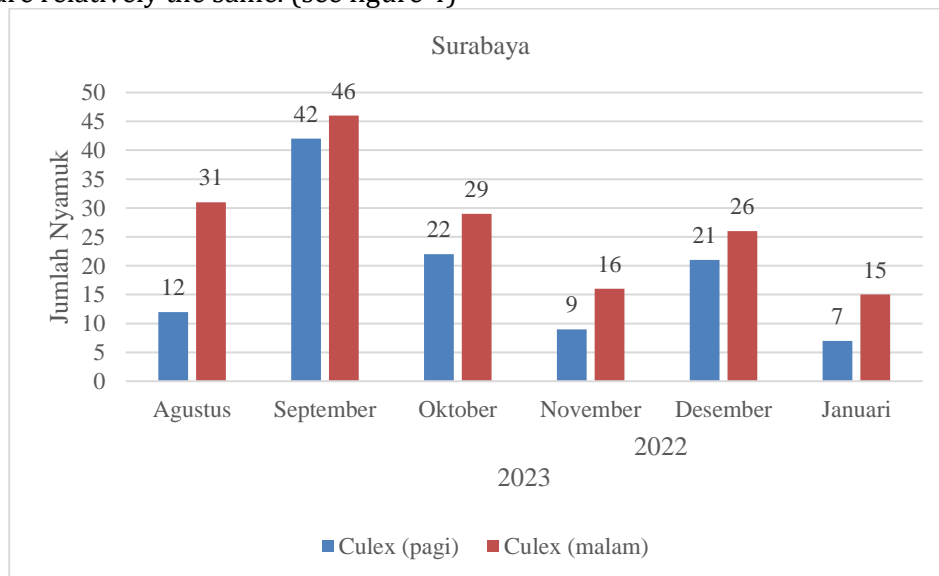


Figure 4 Distribution of Culex spp. in Surabaya and BMKG Data

Distribution of Culex spp. mosquitoes. in Surabaya for 6 months

Distribution of Culex spp. mosquitoes. The highest was found indoors at night with 92/277 (33.2%) and the lowest outdoors in the morning with 41/277 (14.8%). Culex mosquito spp. Males were more likely with 142/277 (51.3%). (see table 1)

Table 1 Distribution of Culex spp. mosquitoes. in Surabaya for 6 months

Mosquito distribution	Tenggilis		Kenjeran		Sawahan		Total	
	Female	Male	Female	Male	Female	Male		
Day	Indoor	4	9	13	16	14	17	73
	Outdoor	5	5	1	10	16	4	41 (41,2%)
Night	Indoor	13	3	22	23	16	15	92
	Outdoor	11	15	8	8	12	17	71 (58,8%)
Total	33	32	44	57	58	53	277 (100%)	

Distribution of insecticide use

The distribution of insecticide use was obtained through questionnaires distributed at the study site. The total number of questionnaires distributed was 60, with 20 questionnaires distributed in each region.

People in Tenggilis, Kenjeran, and Sawahan sub-districts use Baygon the most, with 8/20 (40%), 11/20 (55%), and 7/20 (35%) respectively. People in Tenggilis sub-district who do not use insecticides amount to 1/20 (5%). People in Kenjeran and Sawahan sub-districts use mosquito nets and Autan at least 1/20 (5%) and 2/20 (20%) respectively. (see table 2).

Table 2 Distribution of Insecticide Use

		Insektisida		n = 20	
		Kind	Content	Σ	%
Tenggilis	Baygon		Sipermetrin 0.1%	8	40%
			Imiprotrin 0.031%		
			Pralettrin 0.03%		
	HIT	Transflutrin 25%	5	25%	
VAPE		Dimeflutrin 0.04%	6	30%	
		Pralettrin 0.12%			
		Siflutrin 0.02%			
		Not disposable		1	5%
		Insektisida		n = 20	
		Jenis	Kandungan	Σ	%
Kenjeran	Baygon		Sipermetrin 0.1%	11	55%
			Imiprotrin 0.031%		
			Pralettrin 0.03%		
	HIT	Transflutrin 25%	7	35%	
Mosquito coils	Dimeflutrin 0.05%	1	5%		
		Non-insektisida			
		Kelambu		1	5%
		Insektisida		n = 20	
		Kind	Content	Σ	%
Sawahan	Baygon		Sipermetrin 0.1%	7	35%
			Imiprotrin 0.031%		
			Pralettrin 0.03%		
	HIT	Transflutrin 25%	4	20%	
Mosquito coils	Dimeflutrin 0.05%	4	20%		
Force Magic		Pralettrin 0.09%	3	15%	
		Permetrin 0.15%			
Autan		Diethyltoluamide	2	10%	
		(DEET) 15%			

5 Geographic Distribution of VGSC and ace-1 gene mutations in Culex spp.

Geographic distribution of VGSC and ace-1 gene mutations in Culex spp. in Surabaya can be seen in figure 5.

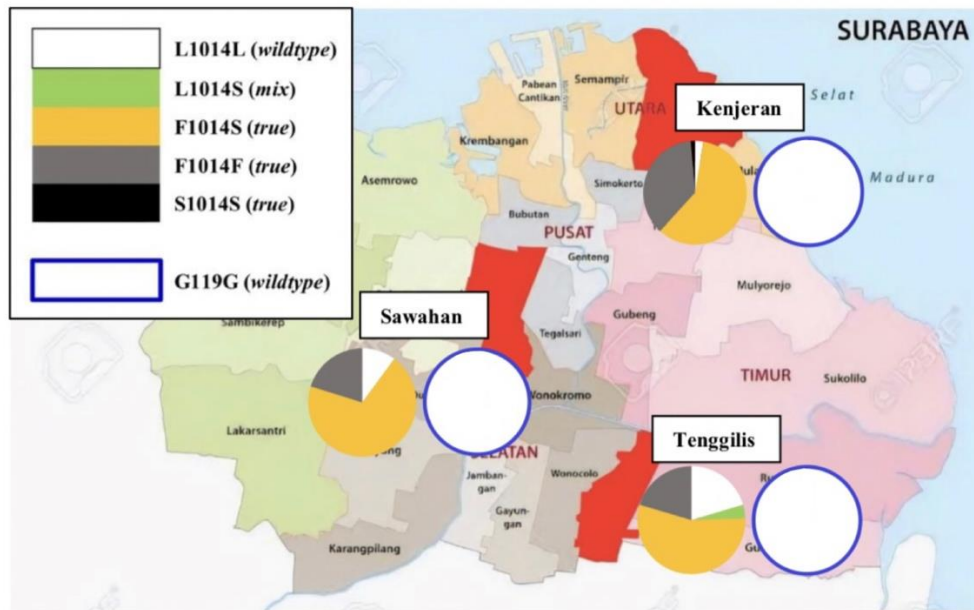


Figure 5 Geographic Distribution of VGSC and ace-1 gene mutations

Mutation Variation of VGSC Gene in Culex spp. in Surabaya

Mutational variation of the VGSC gene in Culex spp. in Surabaya consists of wildtype (LL), heterozygous (LS, FS), and homozygous (FF, SS) genotypes. Wildtype (LL), heterozygous (FS), and homozygous (FF) genotypes were detected in Surabaya with numbers being 21/214 (9.8%), 134/214 (62.6%), and 56/214 (26.2%), respectively. Heterozygous genotype (LS) was only detected in Tenggilis sub-district with a number of 2/214 (0.9%). The homozygous genotype (SS) was only detected in Kenjeran sub-district with a number of 1/214 (0.5%). Heterozygous genotype (LF) was not detected in Surabaya. (see table 3)

Table 3 Mutation Variation of the VGSC Culex spp.

Location	n	Genotype VGSC						Allele Frequency			PHWE
		L1014						L	F	S	
		LL	LF	LS	FS	FF	SS				
Tenggilis	49	10	0	2	27	10	0	0,22	0,48	0,30	0,20
Kenjeran	76	2	0	0	45	28	1	0,03	0,66	0,31	0,00
Sawahan	89	9	0	0	62	18	0	0,10	0,55	0,35	0,00
Total	214	21	0	2	134	56	1	0,10	0,60	0,30	0,00

$P \leq 0,05$: Signifikan (Uji chi square)

Approximation of VGSC Gene Accumulation in Culex spp. in Surabaya

The estimated stages of mutation accumulation in the VGSC gene codon 1014 of Culex spp. mosquitoes in Surabaya can be seen in figure 6 and table 3.

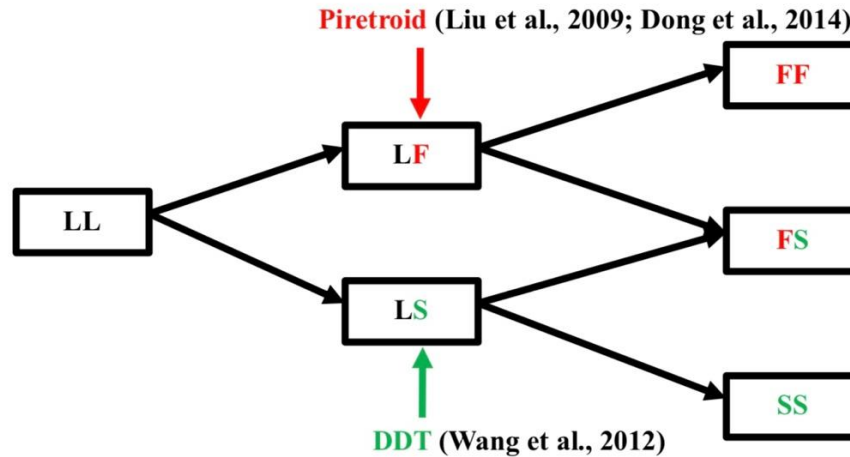


Figure 6 Estimated Stages of Mutation Accumulation of VGSC Culex spp.

Variation of ace-1 gene mutation in Culex spp. in Surabaya

Variation of ace-1 gene mutation in Culex spp. in Surabaya consists of wildtype (GG) genotypes. Wildtype genotype (GG) was detected in Surabaya with a number of 152/152 (100%). Heterozygous (GS) and homozygous (SS) genotypes were not detected in Surabaya. (see table 4)

Table 4 Mutation variation of the ace-1 Culex spp gene.

Location	n	ace-1		
		119		
		GG	GS	SS
Tenggilis	50	50	0	0
Kenjeran	52	52	0	0
Sawahana	50	50	0	0
Total	152	152	0	0

Co-occurrence of VGSC and ace-1 gene mutations in Culex spp.

The variation of co-occurrence of VGSC and ace-1 gene mutations in Surabaya consists of wildtype (LLGG), heterozygous (LSGG, FSGG), and homozygous (FFGG, SSGG) genotypes. Wildtype (LLGG), heterozygous (FSGG), and homozygous (FFGG) genotypes were detected in Surabaya with numbers respectively being 14/139 (10.1%), 99/139 (71.2%), and 23/139 (16.6%). Heterozygous genotype (LSGG) was only detected in Tenggilis sub-district with 2/139 (1.4%). Homozygous genotype (SSGG) was only detected in Kenjeran sub-district with 1/139 (0.7%). Heterozygous genotype (LFGG) was not detected in Surabaya. (see table 5).

Table 5 Co-occurrence of VGSC Gene Mutations and ace-1 Culex spp.

Location	n	VGSC					ace-1
		1014					119
		LL	LS	FS	FF	SS	GG
Tenggilis	41	9	2	25	5	0	41
Kenjeran	48	1	0	36	10	1	48
Sawahana	50	4	0	38	8	0	50
Total	139	14	2	99	23	1	139

Discussion

The distribution of Culex spp. mosquitoes can be influenced by environmental factors. Mosquitoes mainly breed in places with standing water, such as sewers. Human activities such as poor sanitation levels and careless disposal of used goods can also result in the creation of new larval habitats so that the number of mosquitoes can increase (Fagbohun, Idowu, Awolola, & Otubanjo,

2020). *Culex* spp. mosquitoes are more numerous at night compared to during the day. *Culex* spp. mosquitoes are mostly inactive and mostly rest in dark places, such as corners of rooms or shelters during the day (Rani, Surendran, & Thatheyus, 2020). *Culex* spp. is found higher indoors when compared to outdoors. Influencing factors are biting activity by female *Culex* spp. indoors (Syahrani et al., 2022), human activities that generate body heat, and the presence of open water storage (Seang-Arwut et al., 2023). *Culex* spp. males are more abundant than females due to low wind speed factors (Seang-Arwut et al., 2023) and the presence of foods containing sugar as an energy source (Peach et al., 2020). The high mosquito of male *Culex* spp. can be caused by low wind speed and the presence of food sources.

The highest population of *Culex* spp. mosquitoes was obtained in September 2022. Climatic conditions in that month are optimal for mosquito breeding. The optimum temperature and humidity for mosquito development are 22-32°C and >60% respectively. (Ahmed, Hyder, Liaqat, & Scholz, 2019), while the lowest *Culex* spp. mosquito population was obtained in January 2023. The rainfall in January is higher (281 mm) than in other months. High rainfall affects the quality and quantity of mosquito breeding sites. High rainfall also causes larvae from mosquitoes in the breeding site to be wasted or eliminated so that the number of adult mosquitoes can be reduced (Drakou et al., 2020). The low population of *Culex* spp. mosquitoes in Surabaya is caused by heavy rainfall in January 2023.

Pyrethroid class insecticides are widely used by people in Surabaya. Pyrethroids are found in many products circulating in the community. Pyrethroids are chemical insecticides that have a target action in the VGSC gene. The gene plays a role in controlling impulses and regulating membrane excitability through sodium channels (Dong et al., 2014). The mechanism of action of pyrethroids is to disrupt sodium balance so that normal nerve impulses are disrupted and cause hyperexcitation (Cuervo-Parra, Cortés, & Ramirez-Lepe, 2016). Pyrethroids can be associated with the incidence of mutations in codon 1014 of the VGSC gene (Rai & Saha, 2022). The behavior of people in Surabaya in the use of pyrethroid household insecticides can cause mutations in the VGSC gene codon 1014 *Culex* spp. mosquitoes.

Mutation variations of the VGSC *Culex* spp. gene show that there are three genotypes, namely wildtype (LL), heterozygous (LS, FS), and homozygous (FF, SS). Heterozygous (FS) and homozygous (FF, FS) genotypes caused by point mutations in codon 1014 of the VGSC gene are found in the *Culex* spp. mosquito population in Surabaya. Heterozygous (LF) genotype was not detected in the *Culex* spp. mosquito population in Surabaya. Another study in Nigeria showed that homozygous/FF genotypes (45.1%) were more prevalent than heterozygotes/LF (36.3%) and wildtypes/LL (18.6%) (Okafor et al., 2023). Another study in Senegal showed that wildtype/LL genotypes (53.3%) were more common than heterozygotes/LS (27%), LF (6.8%), FS (3.6%), and homozygous/FF (4.9%), and SS (4.4%) (Diallo et al., 2021). Another study in Korea showed that homozygous/FF genotypes (43.6%) were more prevalent than heterozygous/CF (24.9%), LF (16.9%), wildtype/LL (11.5%), and homozygous/CC (3.1%) (Park et al., 2020). Heterozygous (CF) and homozygous (CC) genotypes are variations not found in the *Culex* spp. mosquito population in Surabaya. Another study in Thailand showed that wildtype/LL genotypes (67.1%, 289/431) were more abundant than heterozygous/LF (14.8%, 64/431) and homozygous/FF (18.1%, 78/431) (Chamnanya et al., 2022). The L1014F/S mutation has been reported to have high resistance to DDT and pyrethroid insecticides in *Culex quinquefasciatus* mosquitoes (Liu et al., 2009; Dong et al., 2014) and L1014S have a high resistance relationship to DDT (Wang et al., 2022). The stages of accumulation of VGSC gene mutation codon 1014 of *Culex* spp. mosquitoes The homozygous (FF) genotype of *Culex* spp. mosquitoes obtained in Surabaya is still lower than Nigeria (Okafor et al., 2023) but higher than Senegal (Diallo et al., 2021) and Thailand (Chamnanya et al., 2022) so vigilance is needed against the use of pyrethroid insecticides.

Variations of the *ace-1* gene mutation show that only one genotype is obtained, namely wildtype (GG) with a number of 152/152 (100%). Heterozygous (GS) and homozygous (SS) variations were not detected in Surabaya. Another study in Korea showed results of 99/100 (99%) wildtype

(GG) and 1/100 (1%) heterozygous (GS) (Jeon, Ryu, & Choi, 2024). Another study in the People's Republic of China showed that wildtype (GG) genotypes were found to be the least comparable to heterozygous (GS) and homozygous (GG) with numbers of 18/312 (5.8%), 103/312 (33%), and 191/312 (61.2%) respectively (Feng et al., 2015). Another study in Vietnam showed that wildtype (GG) genotypes were found to be the least compared to heterozygotes (GS) and homozygotes (GG) with numbers of 6/202 (3%), 95/202 (47%), and 101/202 (50%) respectively (Lin et al., 2020). Mutations in the *ace-1* gene are not associated with the use of pyrethroid insecticides but organophosphates and carbamates. The detected variations indicate that organophosphates and carbamates are still sensitive to *Culex* spp. mosquitoes (Essandoh et al., 2013). The absence of mutations in the *ace-1* codon 119 gene of *Culex* spp. mosquitoes in Surabaya shows that organophosphate and carbamate insecticides are still sensitive.

The VGSC gene in *Culex* spp. mosquitoes in Surabaya has undergone mutations. Mutations in codon 1014 indicate that *Culex* spp. mosquitoes are not sensitive to pyrethroid insecticides. *Culex* spp. can be a vector of JEV and filariasis so that the control of *Culex* spp. mosquito vectors needs to be considered, namely using insecticides instead of pyrethroid groups (organophosphates and carbamates). Further and periodic monitoring needs to be done for the evaluation of insecticide resistance.

The weakness of the study conducted is little or lack of data from previous studies on the distribution and mutation of VGSC and *ace-1* genes in *Culex* spp. mosquitoes in Surabaya. The method used in the DNA extraction process is Chelex. This method requires high skills and the results obtained are not good. The questionnaire cannot be used in statistical tests to look for associations between insecticide use and mutations in the VGSC and *ace-1* *Culex* spp. genes in Surabaya because the number of questionnaires and sample points are not comparable.

4. Conclusion

The genotypes of VGSC gene mutations in codon 1014 in *Culex* spp. mosquitoes in Surabaya were LL (9.8%; 21/214), LS (0.9%; 2/214), FS (62.6%; 134/214), FF (26.2%; 56/214), and SS (0.5%; 1/214). The genotype of the *ace-1* gene mutation in codon 119 in the *Culex* spp. mosquito in Surabaya is wildtype GG (100%, 152/152). Genotype mutation in codon 1014 of the VGSC *Culex* spp. gene:

LL(20.4%; 10/49), LS (4.1%; 2/49), FS (55.1%; 27/49), and FF (20.4%; 10/49) were found in the *Culex* spp. mosquito population in Tenggilis sub-district

LL (2.6%; 2/76), FS (59.2%; 45/76), FF (36.9%; 28/76) and SS (1.3%; 1/76) were found in the mosquito population of *Culex* spp. in Kenjeran sub-district

LL (10.1%; 9/89), FS (69.7%; 62/89), and FF (20.2%; 18/89) were found in the *Culex* spp. mosquito population in Sawahan sub-district.

The wildtype genotype on codon 119 of the *ace-1* (GG) gene was found in the mosquito population of *Culex* spp. in the sub-districts of Tenggilis, Kenjeran, and Sawahan.

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