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Inheritance of insecticide resistance to permethrin in *Aedes aegypti* (Diptera: Culicidae)

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Abstract
Aedes aegypti has been resistant againts Permethrin insecticide, generally the insecticide resistance development caused by internal factor as genetics. This study was designed to elucidate the mode of inheritance of resistance to permethrin in *Ae.aegypti*. Bioassay test was conducted to six *Ae. aegypti* field strains (Ant^{G0}, $Skjt^{G0}$, $Skpd^{G0}$, $Bteng^{G0}$, $Bbat^{G0}$, and $Rncs^{G0}$) to determine the development of resistance to Permethrin. The results showed that in six field strains, the offspring responses to Permethrin were different and reflected their resistance ability. Reciprocal crossing between resistant parental strain (Ant^{G9}) with parental susceptible strain (VCRU) resulted in logarithmic concentration vs probit response of their offspring which were similar to those of the susceptible strain, with degree of dominance values (D) -0.2 (when susceptible parent was male in F1) and -0.1 (when resistant parent was female in F1'). The present study suggested that inheritance of Permethrin resistance was incompletely recessive and was autosomal inheritance or has no maternal effect. Permethrin resistance in *Ae. aegypti* was apparently being controlled by one gene.

Keywords: *Aedes aegypti*, inheritance, resistance, permethrin

1. Introduction
Aedes aegypti (L) is the main vector of dengue fever (DF) and dengue hemorrhagic fever (DHF) which are still being serious public health problem until now. The DHF has been a health problem for more than four decades in Indonesia. Since detected of DHF for the first time in Surabaya and Jakarta at 1968, several dengue outbreaks have been reported. For example in 2010, Indonesia's Ministry of Health in 2010 recorded 150,000 cases with 1,317 deaths. Furthermore, in 2016, 201,885 cases with 1,585 deaths were reported [1]. The prevention of the widespread of this disease is still depending on insecticide to control the population of the mosquito vector. The most used insecticide to date comes from synthetic insecticides class Pyrethroid such as Permethrin [2]. The dependence on insecticides to control mosquito population and used it continuously has led to the emergence of resistance. The mosquito has been resistant to various classes of insecticides including Pyrethroid in some country in the world. However, several cases were reported from Indonesia, such as *Ae. aegypti* which collected from Bandung, Surabaya, and Palembang provinces had resistant to Organophospate and Pyrethroid [3], *Ae.aegypti* collected in Semarang city had also resistant to Pyrethroid (Cypermethrin) [4]. The development of resistance was caused the frequency of insecticide use and also derived from the genetic factor of the insect. *Ae. aegypti* from Andreas Jamaica strain has been resistant to Permethrin, the resistance mechanism that occurs caused by gene mutations that are homozigous and heterozigous [5]. The present study aimed to obtain the genetic information of the dominance resistance and the pattern of inheritance resistance properties to Permethrin in *Ae. aegypti* (autosomal or monogenic) under controlled condition at the laboratory. The information on the pattern of inheritance of insecticide resistance properties is very useful for detection and monitoring of resistant insects and the development of resistant insect management strategies [6].

2. Materials and Methods
2.1 Mosquitoes
The mosquitoes used in this study were an *Ae. aegypti* species consisted of six field strains (Ant^{G0}, $Skjt^{G0}$, $Skpd^{G0}$, $Bteng^{G0}$, $Bbat^{G0}$, and $Rncs^{G0}$) that were collected from six regions in the city of Bandung (Antapani, Sekejati, Sukapada, Bandung Tengah, BuahBatuand Rancasari) and susceptible strains (VCRU) has available in the laboratory (from Universiti Sains Malaysia).The next generation of each field strain was selected with Permethrin insecticide to get relatively high resistance level of offspring if compared from the previous generation. A strain that has a relatively high lethal concentration (LC_{50}), resistance ratio (RR_{50}), and survival percentage were used as resistant parental strains. The larvae and adult mosquitoes were maintained at room temperature (25−27^0 C), relative humidity (58−70%) and photoperiod 12:12.

2.2 Biological selection test
The Permethrin stock (92%, obtained from Inti Everspring Indonesia Company) was dissolved in acetone. The mosquito larvae bioassay conducted using a WHO standard test method (WHO, 2005), each bioassay consists of 10−25 individuals of early fourth instars larvae in150−250 ml of water and 1% of Permethrin solution in acetone in each concentration. The

concentration given was four concentrations from 0 %< mortality <100 %.The control group was given only 1% of acetone. Every treatment was replicated as much as four times. The mortality of larvae was calculated after 24 hours of treatment (Li and Liu, 2010).The selection procedure used in the six field strains in each generation was based on the LC_{50}values before and after selection. After that, the test proceeds with a biological test on off spring of each strain to determine the resistant strains that will be used as parental resistant strains. The Antapani strain (Ant^{G0}) were selected with permethrin until nine generations and resulting Ant^{G9} strain (parental resistant strain) with higher resistance levels than Ant^{G0} (parental strain).

2.3 Genetic crossing

The Ant^{G9} resistant strain crossed reciprocally with VCRU susceptible strain using reciprocal crossing method [7]. The male and female mosquitoes were used on each reciprocal cross as many as 50 pairs. The crosses were susceptible female crossed with resistant male and resistant females crossed with susceptible male. The virgins male and female were used on both reciprocal crosses obtained with isolate the pupa individually. The offspring of susceptible females and resistant males were called F1 hybrid, where as the offspring of a resistant female and susceptible male called F1' hybrid. Some of the population was used to biological test and another was reared to adults for the backcross test purposes with parental susceptible parental strain [7, 8, 9].

2.4 Data Analysis

The mortality value of biological test for each generation and each field strains were analyzed with Probit analysis using computer program POLO to get the values of LC_{50} [10]. The resistance ratio was obtained from comparison between LC_{50} values of resistant strains with LC_{50} of susceptible strains. The resistance ratio was grouped into five categories [12], as the following:

$RR_{50} \leq 1$: not resistant
$1 < RR_{50} \leq 5$: low resistance
$5 < RR_{50} \leq 10$: medium resistant
$10 < RR_{50} \leq 50$: high resistance
$RR_{50} > 50$: very high resistance

A resistance dominance property (D) on offspring of the reciprocal hybrid was calculated using the formula [12] as follow:

$$D = \frac{2Xb - Xa - Xc}{Xa - Xc}$$

D = level of dominance, Xa = $\log_{10}$ [LC_{50}] resistant population, Xb = $\log_{10}$ [LC_{50}] heterozygous population, and Xc = $\log_{10}$ [LC_{50}] susceptible population. If a value of D = -1then the inherited resistance was recessive, if the value of D = 0 then resistance was intermediate, and if the value of D = 1 then the resistance is dominant.

Resistance inheritance monogenic properties determine from chi-square value of death and life of offspring in backcross test [13]. The deviation value of χ^2 for all concentration test at a concentration-response line totaled and significance tests using n-2, with n is the number of test concentrations in the concentration-response line [15]. If the value of χ^2 was smaller than the value of χ^2 tables then the resistance character is controlled by one gene (monogenic) [7, 13].

3. Results & Discussion

3.1 Preliminary bioassay and LC_{50} determination of early generations of mosquitoes

The LC_{50} values of VCRU susceptible strains were low and significantly difference to LC_{50} values of field strain against Permethrin in the toxicity test. The status of resistance each strains from high, medium and low categories were $Bteng^{G0}$, $Skjt^{G0}$, $Rncs^{G0}$,Ant^{G0}, $Skpd^{G0}$, and $Bbat^{G0}$ with values 26, 10, 8, 6, 5 and 4 respectively (see Table 1).

The lowest slope value was in $Bteng^{G0}$ strain (1.5) with high resistance level and has the highest value of RR_{50} than other field strains. The heterogeneity level of $Bteng^{G0}$ strain still higher than the other field strains or the proportion of resistant individuals in population was still low although overall the number of resistant individuals.

The value of the VCRU susceptible strain was relatively high (1.9), this indicates that all individuals in the population have similar or homogeneous at susceptibility levels. Therefore, VCRU susceptible strain used as parental susceptible strains in the test of resistance inheritance to Permethrin. The VCRU susceptible strain has been used as the susceptibility standard because it was rearing in the laboratory without insecticides exposure over three decades [15].

Table 1: The toxicity of Permethrin in VCRU susceptible strain larvae compared with field strain larvae in early generation

Strains	Generation	n	Mortality (%)	LC_{50} (ppm)**	Slope± SE	RR_{50}
Ant	G0	200	46	0.29 (0.22-0.39)^{c}	1.83±0.30	6.40
Skjt	G0	320	32	0.46 (0.32-0.64)^{c}	1.85±0.30	10.10
Skpd	G0	200	52	0.26 (0.21-0.31)^{c}	3.14±0.30	5.60
Bteng	G0	400	40	1.19 (0.92-1.64)^{e}	1.53±0.30	26.40
Bbat	G0	300	55	0.19 (0.16-0.23)^{c}	2.91±0.30	4.30
Rncs	G0	200	37	0.37 (0.29-0.52)^{d}	2.01±0.30	8.20
VCRU	-	200	78	0.04 (0.02-0.06)^{a}	1.91±0.40	1*

- FL = Fiducial limit was the lower limit and upper limit of permethrin concentration, insecticidal toxicity was significantly different when the 95% confidence interval limit does not overlap [16]
- * was considered 1 as a comparison
-** Different letters in the same column was significantly different

3.2 Advanced bioassays and selection of mosquitoes strains

Six field strains were selected with advanced bioassays against Permethrin under controlled condition in the laboratory. The strain with the highest value of resistance ratio (RR_{50}) will be a resistant parental strain for a test of inheritance resistance of Permethrin in *Ae. aegypti*. The Bioassays selection test on each generation of field strains aim to eliminate the presence of susceptible individuals in resistant populations; it is conducted to determinate the parental resistant strain.

The LC_{50} value in the Ant strain increased from 0.290 ppm (early generation, G0) to 1.010 ppm (first generation, G1) then to 1.772ppm (second generation, G2). The LC_{50} value increased and significantly difference in the third generation (G3) to 5.049 ppm. The LC_{50} value decreased in the fourth generation (G4) to 4.132 ppm and increased again in the fifth generation to 5.740 ppm. A decreased in LC_{50} values on resistance developments per generation has also been reported on *Ae. aegypti* selected by Pyrethroid and organophosphate [17]. It is could be due to a recessive allele that is associated with mutations of KDR *(knockdown resistance)* which is the target site of Permethrin.

The LC_{50} value in the early generation (G0) of Skpd strain was 0.255 ppm, the value increased in the first generation (G1) until 0.468 ppm, in the second generation (G2) until 2.121 ppm and in the third generation (G3) until 6.919 ppm. The LC_{50} value in the fourth generation (G4) decreased to 4.930 ppm. The value of the slope found in the fourth generation (G4) showed that individuals in this population relatively have the same ability to detoxify a toxic compound of insecticides tested. The LC_{50} value also increased in each generation of Skjt strain, the LC_{50} value of early generations (G0) was 0.457 ppm and increased in first generation (G1) to 0.663 ppm. The LC_{50} value was significantly different between the first generation (G1) and second generation (G2) from 0.663 ppm to 5.068 ppm. The LC_{50} value also increased in the third generation (G3).

The LC_{50} value in Bteng strain also increased on each generation. The LC_{50} value on early generations (G0), first generation (G1), second generation (G2) and third generation (G3) were 1.190, 3.153, 6.282 and7.753 ppm, respectively. The LC_{50} value on Bbat strain as well increased in the three generations (G0, G1, and G2) was 0.197, 0.250 and 0.348 ppm, respectively. The LC_{50} increased as the *Ae. aegypti* larvae response to Permethrin and that was a gradual process. The third (G3) and fourth (G4) generation mortality responses resulted LC_{50} values were 1.266, and 1.937 ppm, respectively. The increased of LC_{50} values on each generation of Rncs strain from early generation (G0) to subsequent generations (G1, G2, G3, and G4) respectively 0.371, 0.654, 0.986, 1.941 and 3.021 ppm.

The increasing of LC_{50} value in biological and selection tests of each generation illustrates the resistance speed of insecticides exposure. The LC_{50} value of each generation on all field strains was important to determine the pattern of resistance ratio value (RR_{50}).The resistance properties on the field strain obtained phenotypically occurs because of ability to survive and continue to develop themselves despite exposure to insecticides.

The strain that has a relatively high level of resistance was the fifth generation (Ant^{G5}) of Ant strain reached 194 times (LC_{50}=8.740 ppm) and the fifth generation of Skjt strain ($Skjt^{G5}$) reached 283 times (LC_{50} = 12.745ppm). The Ant strain (Ant^{G5}) has higher survival percentage (62%) if compared to the Skjt strain ($Skjt^{G5}$) (60%), and then Ant strain made as parental resistant strain in reciprocal crossing.

3.3 Permethrin Selection on Antapani Strain

The selection of fifth generation (Ant^{G5}) of Antapani strain (parental resistant) continued to the next generation with Permethrin exposure concentration 8.7 ppm. The selection has done with nine generations to increase the homogeneity of resistant phenotype from the inbreeding process of individuals on each generation.

Table 2: The selection of Permethrin at 8.7 ppm LC_{50} against *Ae. aegypti* (Ant^{G5}) larvae

Generation	Number of selected larvae	Survival rate (%)
G5	527	30.00
G6	541	58.31
G7	650	59.07
G8	300	66.89
G9	300	77.23

The number of larvae used in the selection process on the fifth generation of Antapani strain (Ant^{G5}) as many as 527 larvae. The survival rate of larvae was 30 % after 1% Permethrin exposed for 24 hours. Furthermore, the larvae were selected in the next generation by using the same concentration. The percentage of larvae survival rate after exposed with Permethrin showed the resistance was increased to the next generation (Table 2). The selection process can change the composition of the pre-adaptive factors, so there is a response change of mortality in the next generation.

3.4 Inheritance of Permethrin Resistance Properties in *Ae. aegypti*

The LC_{50} value of reciprocal offspring between susceptible female with resistant male (F1) was 0.34 ppm and not significantly different from the LC_{50}value of reciprocal offspring between resistant female and susceptible male (F1 '), 0.46 ppm (Table 3). Both of LC_{50} values on reciprocal offspring were overlapped. The similarity of LC_{50} values indicates that autosomal effect on the inheritance of Permethrin resistance properties at *Ae. Aegypti* derived from both parents.

The reciprocal mating dominance values (D) of F1 hybrid and F1'were -0.2 and -0.1, respectively (Table 3). The both of dominant values of the reciprocal mating result were in the range of -1 to 0, which means the insecticide resistance in *Ae. Aegypti* not fully recessive.

Table 3: The Permethrin toxicity in VCRU susceptible strains, resistant strains of Ant^{G9}, reciprocal offspring F1 and F1 ' of *Ae.aegypti*

Strains	n	LC_{50} (ppm) FL 95%	RR_{50}	Slope± SD	Dominance value (D)
VCRU (S)	200	0.05 (0.02-0.06)		1.91±0.4	
Ant^{G9} (R)	300	8.74 (7.07-11.46)	194.20	1.90±0.3	
F1(S♀ X R♂)	400	0.34 (0.17-0.50)	7.60	1.65± 0.3	-0.2
F1'(R♀ X S♂)	400	0.46 (0.10-0.84)	10.10	1.10± 0.3	-0.1

FL (Fiducial limit) = The lower limit and the upper limit of Permethrin, insecticidal toxicity was significantly different when 95% confidence interval limits did not overlap [18].

The mortality percentage (80%) relatively same in the reciprocal offspring (F1 and F1') showed the allele of susceptible larvae have been killed by Permethrin and also indicates the resistance was recessive. The *Ae. aegypti* Kaohsiung strain in Taiwan have Permethrin resistant which is inherently recessive [18]. The offspring of reciprocal mating between resistant females with susceptible males (F1') has a resistance ratio more than 10 times compared to the susceptible strain population (VCRU), while the off spring of susceptible females with resistant males (hybrid F1) has resistance ratio more than 7 times compared to VCRU. It indicates the addition of susceptible properties decrease in resistant allele frequencies in the next generations and affect to decrease in resistance ratios of F1 and F1' than a parental resistant strain. If the resistance was recessive then the next offspring will be dominated by susceptible alleles. Then the dominance of susceptible alleles was higher, if that generation was mating with susceptible strain. Such information of the decreasing of frequency of resistant alleles in the reciprocal offspring between resistant and susceptible strains is very useful in resistance management strategies.

The characterization of genes number was involved in the resistance carried out by backcrossing test among reciprocal offspring with their parents. The test did in the backcross offspring (fourth instars larvae) BC_A (F1'♀ X VCRU♂) and BC_B (F1'♂ X VCRU♀). The number of genes were involved in the Permethrin resistance (χ^2) was 4.5 (BC_A) and 6.1 (BC_B) (Table 4). Both result of χ^2 for all test concentrations that used on the backcross test were lower if compared with the χ^2 of table value (23.6) with a degree of freedom (df) values 14. It indicates that resistance to Permethrin in *Ae. aegypti* in Ant strains were controlled by one gene (monogenic). The monogenous factors were involved in the inheritance of resistance in *Ae. Aegypti* against DDT and Permethrin [18], but another study reported that the inheritance of insecticide resistance properties in mosquito strains was controlled by more than one gene (polygenes) [19, 20, 9]. The resistance in *Ae. aegypti* SAN-F14 strain againts Deltamethrin was inheritanced by autosomal and not fully dominant and involved two factors minimal [21].

Table 4: Inheritance resistance properties to Permethrin in *Ae. aegypti*

Strain	n	LC_{50}(95% CL) (ppm)	RR_{50}	Slope	χ^2 (0.05, 14)	χ^2	df
VCRU	200	0.05 (0.02-0.06)		1.9(0.4)			
BC_A(F1'♀XS♂)	400	0.40 (0.11-0.70)	8.9	1.1(0.3)	23.6	4.46	14
BC_B(F1'♂XS♀)	400	0.34 (0.06-0.67)	7.5	1.5(0.3)	23.6	6.11	14

The determination of the parent used in the backcross test was performed after the resistant inheritance of reciprocal offspring known. The backcross test using susceptible parent also did [8] and have different result from some previous studies [9, 22] who used both the parents (resistant and susceptible). The resistance in *Ae. Aegypti* was partially or fully recessive, with its reciprocal offspring tested backcross with parental resistant and vice versa [23]. The number inadequacy of resistant offspring obtained from the parent of the ninth generation of Ant strain (Ant^{G9}) may be linked to each other due to their negative fitness (the resistancy may be found in most females) [22]. The backcross test between reciprocal offspring (F1 and F1') and resistant parent was not performed. The inheritance of Permethrin resistance on *Ae. aegypti* from Bandung was controlled recessively by a single gene and some result showed the autosomal effect.

4. Conclusions

The permethrin resistance development on *Aedes aegypti* from Bandung was relatively fast because it is related to the biological and physiological properties of mosquito larvae. This condition was affected by short generation period and a large number of offspring as well as the frequency of selection pressures provided. Permethrin Resistance on mosquito from Bandung was inherited in recessive, autosomal and controlled by single gene (monogenic).

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