Genetic diversity of *Elaeidobius kamerunicus* Faust. (Coleoptera: Curculionidae) across geographic areas in Indonesia: implications for oil palm sustainability

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Abstract. *Elaeidobius kamerunicus* is a well-known insect pollinator of oil palm. The presence of *E. kamerunicus* directly creates sustainability in oil palm plantations and successfully increase palm oil production in Indonesia. However, recent reports showed that there are morphometric differences across islands that might be due to different selection pressures that might influence the genetic diversity of the weevil. The aim of this research is to study and analyze *E. kamerunicus* genetic diversity from various locations in Indonesia. Samples were taken using a purposive sampling method from post-anthesis male flowers from 16 regions oil palm centers in Indonesia. Insect DNA amplified by PCR technique using 11 SSR primer. The results showed that some of the SSR markers used were polymorphic. The highest percentage of polymorphic loci is the population of Central Kalimantan, West Java, and Papua. The Population of *E. kamerunicus* in Indonesia has a close relationship and most of the population does not result from inbreeding. Phylogenetic tree constructed using DARwin formed three groups containing mixtures of five spatial distribution regions (islands). The high PIC value and the formation groups indicate the high genetic diversity of *E. kamerunicus* in Indonesia.

Keywords: *Elaeidobius kamerunicus*, group, genetic diversity, PIC value, SSR

1. Introduction

At present, *E. kamerunicus* has become the main pollinator of oil palm plantations that replaces assisted pollination practices in Indonesia [1] with the highest frequency of visits compared to other visitor agencies [2] and has succeeded in increasing palm oil production by 75% [3] but the value of fruit set palm (the percentage of female flowers that become fruit in one fruit bunch) has decreased and shows different results in various regions of Indonesia [4]. This is influenced by many things including pollinator conditions and their effectiveness [5].

To understand the adaptation of organisms to the environment and their genetic changes in population information about genetic structures is needed [6]. Genetic diversity is a level of biodiversity that refers to the total amount of genetic variation in the whole species. Genetic diversity guarantees the survival of species because it can offer higher resistance, the magnitude of diversity within a species depending on the number of individuals, the range of geographic distribution, the level of isolation from the population and the mating system [7].
Recent reports showed that there are differences in body size in various locations. Male imago in North Sumatra has size (length: 4.34 mm, width: 1.55 mm), female imago (length: 3.84 mm, width: 1.27 mm) [8]. Male, in Central Kalimantan has a size (length: 3.61 mm, width: 1.63 mm), female (length: 2.91 mm, width: 1.31 mm) [9]. The differences in body size might be due to different selection pressures that might influence the genetic diversity of the weevil.

One way to find out molecular genetic diversity in a population is to use microsatellite as a marker developed to study population genetics and genetic diversity in insects with high levels of acceptance [10]. Microsatellites are genetic markers used to understand marital systems and structures in a population, linkage, chromosome mapping and population analysis [11]. This marker is known as Simple Sequences Repeats (SSRs) and Short Tandem Repeat (STR) - simple sequences that are less than 100 base pairs, are neutral, polymorphic and repetitive [12]. Microsatellite DNA is an area that has a very high polymorphism. We can find out the variation and structure of a species's population by amplifying and screening, microsatellite also used to determine whether microevolution is occurring leading to speciation [13].

Genetic diversity is a level of biodiversity that refers to the total amount of genetic variation in the whole species. Genetic diversity guarantees the survival of species because it causes variations between individuals. Large genetic diversity can offer greater resistance to diversity within a species depending on the number of individuals, the range of geographic distribution, the level of isolation of the population and the mating system [7].

Genetic diversity in a population can be seen based on the value of its heterozygosity, which describes differences in variation between individuals. This value obtained from the calculation of gene frequency at each locus [14]. Polymorphic locus will describe heterozygosity, the presence of polymorphisms indicates that there is a specific trait of a population [15]. The Aim Research to study and analyze E. kamerunicus genetic diversity from various locations in Indonesia based on SSR markers.

2. Materials and methods

2.1. Insect materials

The samples used in this study were obtained from 16 different locations, namely: Aceh, North Sumatra, Riau (2 collection locations), South Sumatra, West Kalimantan (2 collection locations), Central Kalimantan (2 collection locations), East Kalimantan, West Sulawesi, Central Sulawesi, Banten, West Java (2 collection locations) and Papua. Collection of samples by applying a purposive sampling technique.

Weevils taken from oil palm male flowers post anthesis from several trees with the characteristics of the flowers have turned brown but are still moist and inside there are pupae and larvae. Flower bunches are cut and then put into a transparent sack and left for 48 hours until an adult weevil appears. Imago that appears is collected, stunned by inserting them into the freezer for 30 minutes. Furthermore, males and females separated and samples inserted into insect bottles, labeled and stored in the freezer.

2.2. Isolation of genomic DNA and PCR amplification

DNA was isolated from 20 male and 20 female (separate) insect samples; this number has been optimized to obtain the expected DNA concentration. The isolation process begins by grinding the beetle's body using micropestle. DNA was isolated using the gSYNC™ GS 100-Genaid DNA Extraction Kit and the process was carried out according to the manufacturing instructions delivered. Then the results of DNA isolation were measured using a 2000-Thermo Scientific NanoDrop spectrophotometer to determine the quality and amount of DNA (concentration and purity of DNA).

Microsatellite loci were amplified by Polymerase Chain Reaction (PCR) using eleven primers (table 1). The temperature and time took when DNA amplification was pre-denatured at 95°C for 3 minutes, followed by 35 cycles of denaturation 95°C for 15 seconds, an annealing temperature of 55°C
for 15 seconds, and temperature intensity of 72°C for 15 seconds and then the final elongation process at 72°C and for storage at 4°C. This amplification process uses Thermal Cycler 96 Well-Verity.

2.3. DNA fragment analysis

This analysis is carried out for amplified DNA samples starting with preparing the reagent and working according to the manufacturer's instructions. Each DNA sample was inserted into a well as much as 8 μl and added a Dilution buffer of 16 μl (1 last well was filled with 24 μl 100 bp DNA Ladder). The solution in the well was homogenized (in the vortex) for 2 minutes and centrifuged at a speed of 3000 rpm for 2 minutes. In different wells, 1 ml of the buffer inlet was homogenized and centrifuged. All wells are inserted into the machine fragment analysis according to space provided by the sequence Buffer, Waste, Marker, DNA samples.

The volume of reagents in each reagent bottle in the machine must be considered and adjusted to the recommended level (gel: ≤ 40 ml, conditioner: ≤ 40 ml, waste: empty). The Analysis Process This DNA fragment uses Capillar's Automated CE System – 96 Fragment Analyzer. DNA sequences that have gone through the fragment analysis stage will produce data on the number of base pairs in each DNA sample in each primary locus. This value then becomes the data used to test genetic diversity further, so that it can examine in more detail the structure of the sample population, heterozygosity, polymorphism and genetic proximity analysis between populations.

Table 1. Details of SSR primer sequences used in the genetic diversity analysis.

| Primer Name | Forward Primer (5’-3’) | Reverse Primer (5’-3’) |
|-------------|------------------------|-----------------------|
| SSR12       | AGATGGACGTTCATACATTGGC | GAACGAAGAACTGTGGTGCC  |
| SSR14       | GGTTCACAAACAGCATCGGG  | TTATCTCCCGAAGCGACTGC  |
| SSR16       | TCGTTGATCCTCTCGTCGG   | GCTGGAGATCGTCGTTAAGC  |
| SSR17       | CTTCGGGTGTCTTTAAAAAGGG| TGTATATAGAGGTAGATAATCGAGCC |
| SSR22       | TGGACGACTCCGTITTTTC   | GCGGAATCTACGTACGTACGC |
| SSR42       | CAATTCAGCAGCGGATTG GCC| TTATGCGCACATCAAGCTCC |
| SSR6        | AACATCAGGCGAGGATCG    | TGTATAGCATCCATCGAACAGG |
| SSR137      | AAAAACACTACGGAGTACGC  | GGGTCTCAAGGCGATTGCC  |
| SSR159      | GCTCAGCTTTTACAATAATCGTCC | GTGGAGATATACAGGAGGGGG |
| SSR208      | TGATTTTATCATGGCAGGTGGC| GGACCAAAACAGCAATGGCC |

2.4. Statistical analysis

The genetic parameters of each primer used such as the number of alleles (N), observed heterozygosity values (Ho), expected heterozygosity (He), and Polymorphic Information Content (PIC) loci were computed using the Cervus 2.0. The GenAlEx 6.501 software was used to calculate average allele (Na), average effective allele (Ne), Ho and He population. Phylogenetic tree constructed using DARwin program version 6.05 (Dissimilarity Analysis and Representation for windows).

3. Results and discussion

3.1. Number of alleles and PIC values

Based on the number of alleles per loci (table 2) there are four monomorphic primers, i.e., SSR16, SSR56, SSR137, SSR208 indicated by only one allele in one population. Conversely, there is a locus that has more than one number of alleles which describe that the locus is a polymorphic locus, namely SSR12, SSR13, SSR17, SSR27, SSR42, SSR159. With the presence of polymorphic loci, the population shows value polymorphic higher than other populations can also be studied further. The population with the highest number of polymorphic is 100% of Central Kalimantan, Java, and Papua populations. This means that all detected loci show more than one number of alleles. In contrast, the population with the lowest number of polymorphic is the population of Central
Sulawesi at 64%. The locus was declared polymorphic if the number of alleles in the population at the locus was more than one with the frequency of alleles less than or equal to 0.95 [16].

The highest percentage of polymorphic (PIC) is at the SSR27 locus with a value of 0.93 while the lowest value is at the SSR16 locus with a value of 0.248. There are three loci with PIC values less than 0.5 (SSR16, SSR 56, SSR 208) which indicate that the locus is less informative, and the other eight loci are included in the very informative category. PIC criteria are included in the low group if the PIC value is smaller than 0.25, the PIC value including the medium category is between 0.25 to 0.5, and is included in the high category if the PIC value is greater than 0.5. PIC values higher than 0.7 indicate [17].

Each locus has a different allele, the total of alleles obtained was 122 alleles (mean 11.09) which ranged from 3 to 26 alleles per locus. The locus with the highest number of alleles was found in SSR27 and SSR12 with 26 alleles and 20 alleles different per locus respectively. In contrast, the locus with the lowest number of alleles was observed in SSR16 and SSR56, each of which had three different alleles per locus. Of the 11 loci studied there were two loci (SSR16 and SSR56) less recommended to be used as markers because they did not meet the minimum number of alleles per locus that could be used in each population, on the other 9 loci especially SSR27 and SSR12 were loci recommended as markers because has more alleles than other loci. This is in accordance with the opinion of [18] which states that to assess the genetic variation between marriages at a minimum there must be four different alleles at each locus.
Table 2. Number of allele each population, PIC and allele each locus.

| Loci  | CC  | WK  | EK  | AC  | NS  | RI  | SS  | WS  | CS  | WJ  | PP  | Allele / loci | PIC   |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----------------|-------|
| SSR12 | 3   | 5   | 4   | 4   | 4   | 7   | 4   | 4   | 4   | 6   | 2   | 20             | 0.868 |
| SSR13 | 4   | 6   | 3   | 3   | 2   | 4   | 2   | 2   | 3   | 5   | 2   | 8              | 0.781 |
| SSR16 | 2   | 1   | 1   | 2   | 1   | 1   | 1   | 1   | 1   | 2   | 2   | 3              | 0.248 |
| SSR17 | 5   | 6   | 4   | 4   | 3   | 6   | 3   | 2   | 2   | 6   | 3   | 12             | 0.832 |
| SSR27 | 6   | 5   | 2   | 3   | 4   | 7   | 4   | 3   | 4   | 8   | 3   | 26             | 0.933 |
| SSR42 | 5   | 5   | 3   | 3   | 3   | 4   | 3   | 2   | 2   | 6   | 2   | 10             | 0.795 |
| SSR56 | 2   | 2   | 2   | 1   | 1   | 2   | 1   | 1   | 2   | 2   | 2   | 3              | 0.427 |
| SSR137 | 2   | 3   | 1   | 2   | 2   | 3   | 2   | 2   | 1   | 3   | 2   | 10             | 0.725 |
| SSR159 | 5   | 5   | 3   | 2   | 3   | 4   | 3   | 2   | 3   | 6   | 3   | 12             | 0.830 |
| SSR192 | 5   | 4   | 3   | 4   | 4   | 8   | 3   | 4   | 1   | 7   | 4   | 12             | 0.874 |
| SSR208 | 2   | 1   | 2   | 1   | 2   | 1   | 1   | 1   | 1   | 2   | 2   | 6              | 0.437 |
| Mean  | 3.7 | 3.9 | 2.6 | 2.6 | 2.6 | 4.3 | 2.5 | 2.0 | 2.2 | 4.8 | 2.6 | 11.1           | 0.705 |
| Polymorphic | 11 | 9   | 9   | 9   | 9   | 8   | 8   | 7   | 11  | 11  | -              | -     |
| Polymorphic (%) | 100 | 82  | 82  | 82  | 82  | 73  | 73  | 64  | 100 | 100 | -              | -     |

CK = Central Kalimantan
WK = West Kalimantan
EK = East Kalimantan
AC = Aceh
NS = North Sumatera
RI = Ria
SS = South Sumatera
WS = West Sulawesi
CS = Central Sulawesi
WJ = West Java
PP = Papua
3.2. Heterozygosity and inbreeding coefficient of population

The observed heterozygosity (Ho) value (table 3) in the population with a range of values are 0.545 - 0.636, indicates that the population of *E. kamerunicus* in Indonesia has a close relationship. Range of values of heterozygosity is zero to one. If the value of heterozygosity is equal to zero, the population measured has a very close relationship and if the value of heterozygosity is equal to one, then the population measured does not have a genetic relationship at all [19]. The high value of heterozygosity shows high genetic diversity. This can occur as a result of random mating.

Mean of value observed heterozygosity (Ho) and the expected heterozygosity (He) (table 3) can be used to estimate the value of inbreeding in a population. The Ho value in this population shows a higher value than the He value. The average Ho value of 0.609 is greater than the He value of 0.526. This value indicates that the mating of the population of *E. kamerunicus* in Indonesia is not inbreeding. The expected heterozygosity usually defined when describing genetic diversity because it is less sensitive to the sample size than the observed heterozygosity. When HO and HE are similar (not significantly different), the crossing in the population is almost accidental. When HO < HE, it is an inbred population. When HO > HE, the random mating system dominates inbreeding in the population [20].

**Table 3.** Observed heterozygosity (HO), expected heterozygosity (HE) and Inbreeding coefficient (F) each population.

| Population       | Ho   | He   | F    |
|------------------|------|------|------|
| Central Kalimantan | 0.636| 0.614| 0.123|
| West Kalimantan  | 0.636| 0.577|-0.021|
| East Kalimantan  | 0.545| 0.500|-0.007|
| Aceh             | 0.636| 0.511|-0.200|
| North Sumatera   | 0.636| 0.511|-0.200|
| Riau             | 0.636| 0.602| 0.002|
| South Sumatera   | 0.591| 0.450|-0.283|
| West Sulawesi    | 0.636| 0.398|-0.617|
| Central Sulawesi | 0.545| 0.386|-0.410|
| West Java        | 0.606| 0.650| 0.231|
| Papua            | 0.591| 0.580| 0.030|
| Mean             | 0.609| 0.526|-0.096|

The average value of inbreeding coefficients of all populations is -0.096. This value (negative) indicates that the potential for inbreeding that occurs in this population is still low. However, if examined by each population, there are some who have positive values (Central Kalimantan, Riau, West Java, and Papua) which indicate that the population has greater inbreeding potential than expected. Inbreeding coefficient (F) is the deviation of the observed heterozygosity of an individual relative to the heterozygosity expected under random mating. F > 0 signifies more inbreeding than expected at random, whereas F < 0 indicates that inbreeding occurred less often than would be expected at random. Note that when a population is small, even random mating can lead to mating between relatives [21].

3.3. Phylogenetic tree

Population grouping (picture 1) based on the spatial distribution area using the Neighbor-Joining Tree method shows that each population not grouped by region (island). There are three groups containing mixtures of five spatial distribution regions (islands). The formation of this group indicates a high genetic exchange in the population of *E. kamerunicus* in Indonesia. Grouping individuals from different locations into one group occurs because these individuals have genetic similarities that caused by the presence of genetic recombination or gene flow, especially in adjacent areas and their
parents are closely related [22]. This indicates a high genetic exchange in the population of *E. kamerunicus* in Indonesia. This genetic exchange creates high genetic variation and supports the survival of *E. kamerunicus* in Indonesia.

As the area of origin of *E. kamerunicus* in Indonesia, Siantar has the closest genetic distance to the population of Riau1-female and WestKalimantan1-male with distances of 0.409 and 0.459. The farthest genetic distance with the population of WestJava1-female and SouthSumatera-male is 0.634 and 0.729.

![Phylogenetic Tree](image)

**Figure 1**. The phylogenetic tree is constructed by Neighbor-Joining Tree, bootstrap 10000 based on 11 microsatellite markers using DARwin 6.0.8.

4. Conclusion

*E. kamerunicus* population in Indonesia high genetic diversity and divided into three groups which consist of a combination of several islands. Most of SSR Primer is polymorphic loci, with three primers included in medium category and eight primers others included in the high grade, which can be used as molecular markers. The genetic relationship of *E. kamerunicus* populations in Indonesia is close and does not have the potential to form speciation.

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