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Identification and Phylogenetic Analysis of *Drosophila melanogaster* based on ITS 2 rDNA Sequences

R Oktarianti[1], A Sholihah[1], D Masruroh[1], S Wathon[1], K Senjarini[1]*
[1]Biology Department, Faculty of Mathematics and Natural Sciences, University of Jember, Indonesia

*senjarini@unej.ac.id

Abstract. The Internal Transcribed Spacer 2 (ITS 2) is a small non-coding region located inside the nuclear ribosomal DNA cluster. ITS 2 sequence variability is widely used in taxonomy and molecular phylogeny. Based on this molecular marker, this study aimed to identify and to construct phylogenetic analysis of *D. melanogaster* from our laboratory. The phylogenetic tree was constructed after analysis on DNA sequence was conducted by encoding its ITS2 using *Unweight Pair Group* (UPGMA) method. The result showed that our *D. melanogaster* wild type and all mutant strains have high similiarity to *D. melanogaster* 28 S ribosomal RNA gene partial sequence (Gene bank Acc GU 597379.1) (identity score up to 99%). Phylogenetic tree showed that *D. melanogaster* plum mutant was closely related to *D. melanogaster* 28 S ribosomal RNA gene partial sequence (Gene bank Acc GU 597379.1), they also belonged to one cluster. Next to this cluster are *sepia* and *clot* mutant, in addition to the wild type and black mutant in one cluster. All the mutants and wild type belonged to one clade. While the vestigial mutant stayed in the different clade.

1. Introduction

Drosophila melanogaster Meigen, also known as fruit flies in the order of Diptera and family Drosophilidae, is commonly used as the animal model for studying many topics of genetics, such as population genetics, linkage and crossing over, sex determination, genetic interactions, chromosomal aberrations, evolution, inheritance trait, phylogentic relationship and molecular genetics [1;2]. It is an ideal model organism for several reasons, i.e.: the smallsize of its body, its shortlife cycle, its abundant number of offspring, and the low cost of maintenance [3]. The wild type *Drosophila* was originaly coming from sub-Saharan Africa, which then, due to human activity, spread throughout the world [4]. The spread in population yield an alleles and phenotype changes as a response to their environment. The environmental adaptation caused the wild type *Drosophila* to produce some strains or mutants [5].

Internal Transcribed Spacer (ITS) is one of the molecular markers broadly used in species identification, phylogenetic analysis and other related studies for eukaryotic organisms [6]. Unlike ITS 1, ITS 2 is commonly used in the phylogenetic reconstruction at the genus and species levels because it has a short sequence (±700 bp) and is relatively easy to amplify [7]. The first exploration of ITS 2 in *Drosophila* species was reported by Schlötterer [8]. Followed by Young and Coleman had successfully characterized a single secondary structure in Drosophilids [9]. These developments open a much wider application of ITS 2 for phylogenetic analysis in insect evolutionary studies.

In *Drosophila*, ITS 2 has generally been found to be a useful marker offering resolution at the species level as well as in the genetically structured systems such as in sibling and subgroup species [8]. Several studies have focused on relationships within the *D. melanogaster* subgroup. The previous research showed that phylogeni reconstruction result in subgrup of *D. melanogaster* based on DNA sequences from four nuclear genes (Adh, Adhr, Gld and Ry) the six species i.e *D. melanogaster, D. simulans, D. teissieri, D. yakuba, D. erecta, and D. orena* are included in the melanogaster subgroup. The other hand three species were outside the melanogaster species subgroup were *D. eugracilis, D. mimetica* and *D. Lutescens* [10]. The Other studies phylogenetic relationships on subgrup secies of *D. melanogaster* based on protein can be divided into two complexes, namely *D. melanogaster* complex consisting of *D. melanogaster, D, simulans, D, mauritiana and D. sechellia*,

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and *D. yakuba* complex consisting of *D. yakuba*, *D. teissieri*, *D. erecta*, *D. Orena* [11]. However, there has been no study focusing on the molecular and phylogenetic analysis of mutants in *D. melanogaster* using ITS 2 marker. Therefore, this study aimed to characterize the molecular as well as the phylogenetic relationships among *D. melanogaster* mutants. We used several mutants obtained from our own collection such as black (bl), sepia (se), clot (cl), vestigial (vg) and plum (pm). The results achieved in this study serve as optimal material for evolutionary studies and knowledge of the phylogenetic relationship, especially at the species level.

2. Methods

2.1. Specimen Collection

The strain of *D. melanogaster* were obtained from the collection maintained in the Genetic Laboratory, Biology Department, Faculty of Mathematics and Natural Sciences, University of Jember, Indonesia. The specimens used in this study were wild type, plum, sepia, clot, black and vestigial. The adults collected were processed for genomic DNA isolation.

2.2. Genomic DNA Extraction and PCR Amplification

DNA extraction was carried out by using salt extraction method with minor modifications [12]. The samples (the whole body *Drosophila*) which were stored at -20ºC for 15 minutes were homogenized in 500 µl of homogenizing buffer by using micro-pestle. The homogenate was then added with 40 µl SDS 40% and 8 µl proteinase K (20 mg/ml), and incubated at 65ºC overnight. The homogenate was then added with 300 µl 6 M NaCl and centrifuged at 12.000 rpm for 5 minutes at 4ºC. The supernatant was transferred to a fresh eppendorf tube, and anequal volume of 100% isopropanol was added, then incubated at -20ºC for one hour. The DNA was then pelleted by centrifuging at 12.000 rpm for 20 minutes at 4ºC. The DNA pellet was washed with ethanol 70%. The DNA pellet was air-dried and dissolved in DNA-se free deionized water.

PCR reaction was carried out in a volume of 50 µl containing 2 µl of the template DNA, 20.5 µl of DNAse free deionized water, 25 µl of PCR Master Mix (Intron, USA), and 2.5 µl of each primer (10 pmol/µl). The ITS 2 primers 5.8F (5 'TGT GAA CTG CAG GAC ACA TG 3') and 28R (5 'ATG CTT AAA TTT AGG GGG TA 3') were used to identify the Drosophila species. The thermal profile used to amplify ITS 2 region from *Drosophila* species was initial denaturation at 94ºC for 5 minutes followed by 35 cycles of denaturation at 94ºC for 60 seconds, annealing at 54ºC for 30 seconds and elongation at 72ºC or 60 seconds and a final elongation at 94ºC for 5 minutes [13]. The PCR amplified DNA fragments were visualized in a 1.5% agarose gel stained with Ethidium Bromide on a UV transilluminator.

2.3. Sequence Data Analysis

The PCR products were then purified using 'PCR Clean-up Gel Extraction NucleoSpin® Extract II' (Promega, USA) following the manufacturer's instruction. Sequencing was done by sending the purified PCR product to 1st BASE Singapore. Sequencing data were analyzed using freely available Bioedit software (TomHall, Ibit Therapeutics) and confirmed through BLAST search (NCBI). The phylogenetic tree was constructed by the Unweight Pair Group Method with Arithmatic Mean (UPGMA) method with a bootstrap test (1000 replicates) in MEGA6 software.

3. Results and Discussion

The length of the *D. melanogaster* genomic DNA for all of strains (wild type (wt), sepia (se), plum (pm), black (bl), clot (cl) and vestigial (vg)), as determined by electrophoresis DNA was larger than DNA ladder (1500 bp and 10000 bp) (Figure 1). The *D. melanogaster* genome is ~180 Mbp in size, which third of was centric heterochromatin [14].

Meanwhile, the PCR amplification of ITS2 region produced some distinct fragments of ~557 bp, ~537 bp, ~541 bp, ~487 bp, ~450 bp, ~487 bp for wild type, sepia, plum, black, clot and vestigial strain respectively (Figure 2). These results are in line with the studies, which examined that the DNA sequence of *Drosophila* ITS 2 was 320-429 bp in length [15;16].

As from sequence analysis, all strains of *D. melanogaster* studied have shown proximity with *D. melanogaster* 28S ribosomal RNA gene partial sequence (Gene Bank Accession Number GU597379.1), with identity score up to 99%. To know the phylogenetic relationships among

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Drosophila mutants, ITS 2 sequences of six *D. melanogaster* strains were selected for constructing an evolutionary tree by UPGMA method with 1000 bootstrap value (Figure 3).

Drosophila is a small fly in the order Diptera that is widely used as a model system in the biological research. The use of *Drosophila* as an animal model has dramatic advances in cell and developmental biology, neurobiology and behaviour, molecular biology, evolutionary and population genetics [1]. More observations have enabled to explore the mechanisms of developmental programs, behaviors and complexity of evolution. There are more than 3000 *Drosophila* species known [17]. Nevertheless, the phylogenetic relationships between most of these species are not resolved and are debated among taxonomists [18].

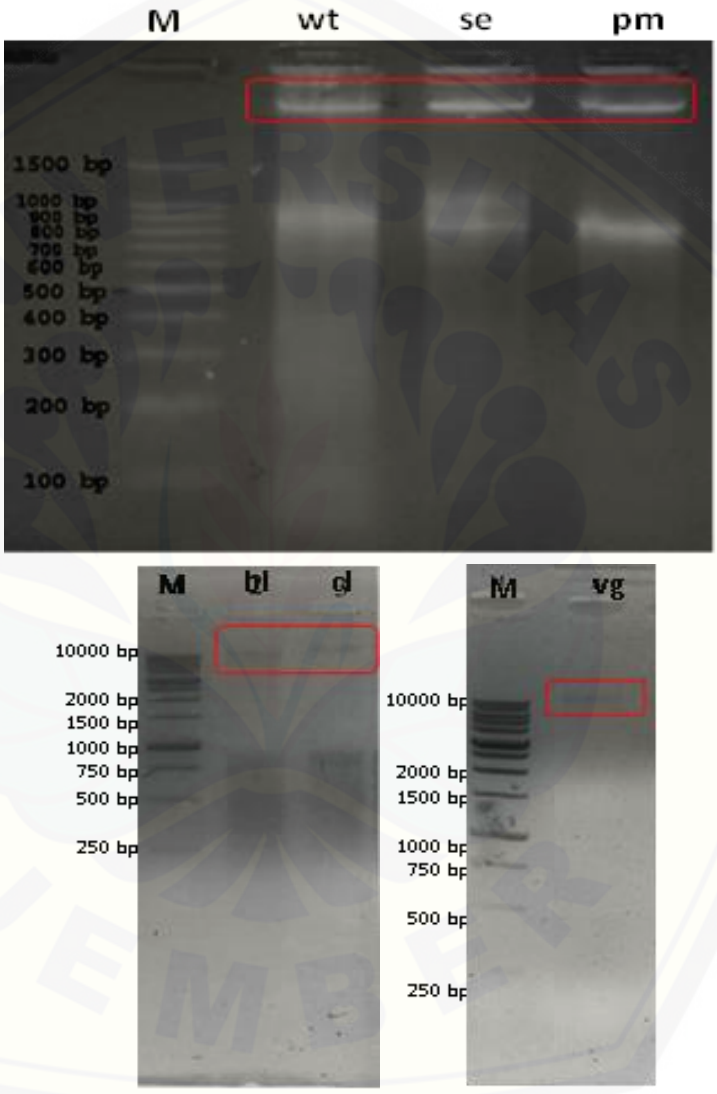

Figure 1. Electroforegram of *D. melanogaster* genomic DNA from all strains. Red line rectangular showed the desired band of genomic DNA.

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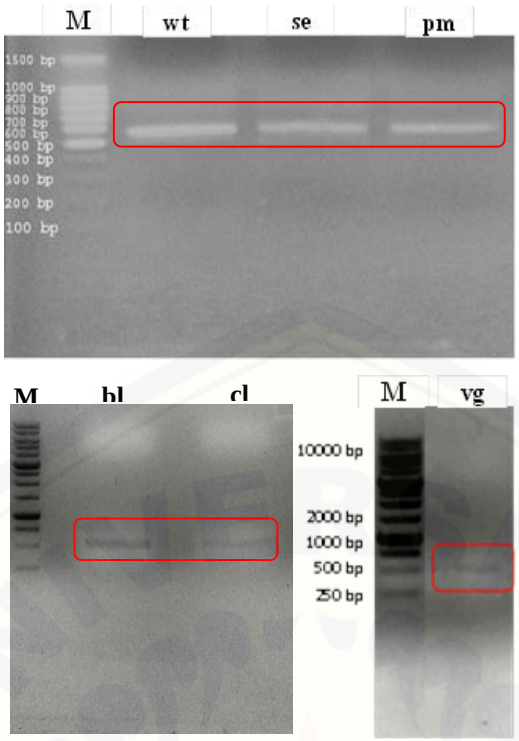

Figure 2. PCR amplification of ITS 2 of *D. melanogaster* from all strains tested. Red line rectangular showed the desired band of ITS 2 DNA.

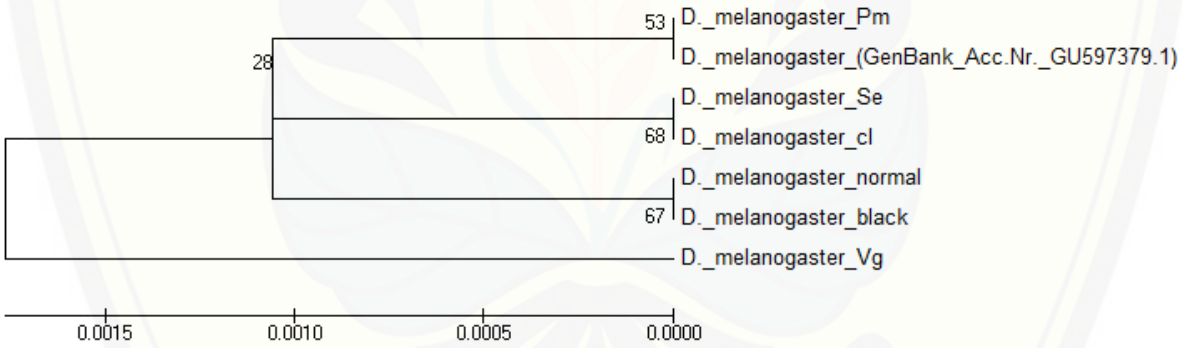

Figure 3. Phylogenetic tree of six *D.melanogaster* strains constructed using UPGMA method. Numbers above branches indicate the boostrap percentage.

DNA barcoding is known as a powerful tool not only for "species identification" but also for "species discovery" or "DNA taxonomy" [8]. Internal Trancribed Spacer (1TS) is one of the markers for DNA barcode used to determine the genetic diversity in eukaryotes. There are two ITS units in each individual, ITS1 located between 18S rRNA and 5.8S rRNA. Otherwise, ITS2 is located between 5.8S rRNA and 28S rRNA [19]. The ribosomal DNA (rDNA) gene family of *Drosophila* species undergoes continual rounds of unequal crossing-over leading to the concomitant spread of mutations [20]. The differences in the nucleotide sequence of ITS region might be caused by point-mutation and structural alterations accumulated in each strains. In this study, we analyzed the molecular phylogenetic of five *D. melanogaster* mutants along with the wild type strains using all the unique ITS 2 sequences of the *Drosophila* group available from this study and from GenBank.

The phylogenetic tree in Figure 3 was constructed using UPGMA method, in which the resulting tree was rooted. The relative positions of *D. melanogaster* strain of wild type, sepia, clot, black and plum were clustered in one clade, whereas the vestigial strain stayed in a different clade. Nevertheless, Figure 3. illustrates that plum mutant is closely related to *D. melanogaster* 28S rRNA gene partial

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sequence (Gene Bank Accession Number GU 597379.1). Vestigial strains are in the different clades because there are many gaps in the nucleotide sequences compared to the other strains. Mutations in vestigial strains occur in wings, whereas strains of clot (chromosome no.2, locus 16.5), plum (chromosome no.2, locus 54.3), and sepia (chromosome no.3, locus 26.0) occur in the eyes. On the contrary, mutations in the black strain (chromosome no.2, locus 48.5) only affected the body color [21]. This results show that although each strain has a mutation on the different organ, chromosome and locus but they still have closed relationships. There is a considerable equation for heterochromatin in chromosom 2 and 3 from *D. melanogaster* [22].

The phylogenetic tree represents the evolutionary history of an organism. In this study, we identified that *D. melanogaster*_GU597379.1, wild type, plum, sepia, clot, black, and vestigial strain are classified as monophyletic group. This indicates that the members are from single ancestral species. This result was supported by Morgan et al. that *D. melanogaster* has undergone a genetic mutation so that it is known that were 85 mutant strain, have phenotypes that deviate from the wild type of *D.* m*elanogaster* [23].

4. Conclusions

The wild type *D. melanogaster* and all mutant strains have high similarity to *D. melanogaster* 28S ribosomal RNA gene partial sequence (Gene bank Acc.Nr. GU597379.1) (identity score up to 99%). The phylogenetic tree showed that *D. melanogaster* plum mutant was closely related to *D. melanogaster* 28S ribosomal RNA gene partial sequence (Gene bank Acc.Nr. GU597379.1). All the mutants and wild type belonged to one clade. While the vestigial mutant stayed in different clade.

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References

[1] Hales KG, CA Korey, AM Larracuente,DM Robert. 2015. Genetic on the fly: a primer on the *Drosophila* model system. *Genetic*. 201: 815-842.

[2] Linde KD, D Houle, GS Spicer, SJ Steppan. 2010. A supermatrix-based molecular phylogeny of the family Drosophilidae. *Genet. Res. Camb*. 92: 25-38.

[3] Jennings BH. 2011. Drosophila-a versatile model in biology & medicine. *Materials Today*. 14:190–195.

[4] Kapopoulou A, SP Pfeifer, JD Jensen, S Laurent. 2018. The demographic history of african *Drosophila melanogaster*. *Genome Biol. Evo*. 10:2338–2342.

[5] Peschel N, C Helfrich-Förster. 2011. Setting the clock - by nature: circadian rhythm in the fruitfly *Drosophila melanogaster*. *FEBS Letters*. 585:1435–1442.

[6] Chen S, H Yao, J Han, C Liu, J Song, L Shi, Y Zhu. 2010. Validation of the ITS 2 region as novel DNA barcode for identifying medical plant species. *Plos One*. 5: 1-8.

[7] Han J, Y Zhu, X Chen, B Liao, H Yao, J Song, S Chen, F Meng. 2013. The short its2 sequence serves as an efficient taxonomic sequence tag in comparison with the full-length its. *BioMed Research International*. 2013:3–9.

[8] Li YF, SY Wen, K Kawai, JG Jian, GH Yao, R. Segawa, MJ Toda. 2012. DNA barcoding and molecular phylogeny of *Drosophila lini* and its sibling species. *Int. J. Evol Biol*. 2012: 1-9.

[9] Marinho MAT, ACM Junqueira, AML Azeredo-Espin. 2011. Evaluation of the internal transcribed spacer 2 (ITS2) as a molecular marker for phylogenetic inference using sequence and secondary structure information in blow flies (diptera: Calliphoridae). *Genetica*. 139:1189–1207.

[10] Ya Ko W, RM David, H Akashi.2003. Molecular Phylogeny of the *Drosophila melanogaster* Species Subgroup. J Mol Evol. 57: 562–573

[11] Lee WH and TK Watanabe. 1987. Evolutionary genetics of the *Drosophila melanogaster* subgroup I. Phylogenetic relationships based on coatings,hybrids and proteins. J. Genet. 62 : 225-239.

Digital Repository Universitas Jember

[12] Gaaib JN, F Nassief, AH Al-assi. 2011. Simple salting – out method for genomic dna extraction from whole blood. *Tikrit Journal of Pure Science.* 16:15–17.
[13] Walton C, P Somboon, SM O'Loughlin, S Zhan, RE Harbach, YM Linton, B Chen, K. Nol an. 2007. Genetic diversity and molecular identification of mosquito species in the *Anopheles maculatus* group using the ITS2 region of rDNA. *Infection, Genetics and Evolution.* 7: 93-102.
[14] Chang CH, AM Larracuente. 2019. Heterochromatin-enriched assemblies reveal the sequence and organization of the *Drosophila melanogaster* y chromosome. *Genetics.*. 211:333–348.
[15] Tautz D, JM Hancock, DA Webb, C Tautz, GA Dover. 1988. Complete sequences of the rRNA genes of *Drosophila melanogaster*. *Mol. Biol. Evol.* 5: 366-376.
[16] Young I and AW Coleman. 2004. The advantages of the ITS2 region of the nuclear rDNA cistron for analysis of phylogenetic relationships of insects: a Drosophila example. *Molecular Phylogenetics and Evolution*. 30: 236–242.
[17] Sato K, R Tanaka, Y Ishikawa, D Yamamoto. 2020. Behavioral evolution of *Drosophila*: unraveling the circuit basis. *Gene.* 11:1–13.
[18] O'Grady PM, R DeSalle. 2018. Phylogeny of the genus *Drosophila*. *Genetics.* 209:1–25.
[19] Bianciardi A, M Boschi, EE Swanson, M Belloni, LG Robbins. 2012. Ribosomal DNA organization before and after magnification in *Drosophila melanogaster*. *Genetics.* 191:703–723.
[20] Freire R, A Arias, J Méndez, A Insua. 2010. Sequence variation of the internal transcribed spacer (ITS) region of ribosomal dna in cerastoderma species (bivalvia: cardiidae). *Journal of Molluscan Studie.* 76:77–86.
[21] Srivastava D and PN Saxena. 2017. Response of *Drosophila* mutans to korean red ginseng, a natural product. *Eduved*. 4:8–18.
[22] Sidorenko DS, IA Sidorenko, TY Zykova, FP Goncharov, J Larsson, IF Zhimulev. 2019. Molecular and genetic organization of bands and interbands in the dot chromosome of drosophila melanogaster. *Chromosoma*. 128:97–117.
[23] Robert.J.Brokers. 2005. *Genetic Analysis dan Principles*. Third Edition McGrow.Hill International edition