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Plant Chromosome and Karyotype Analysis Training for High School/MA/Equivalent Teachers in Samarinda and its Surrounding Areas

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Abstract Increasing biology teachers' comprehension of the principles of genetics is attainable through the implementation of specialized training sessions focused on chromosome and karyotype analysis. In this training, 21 high school biology teachers from Samarinda and Nears participated in a structured training program encompassing three stages. In the initial phase, participants were provided with materials on the fundamental aspects of genetics. Subsequently, in the second phase, hands-on training was imparted, focusing on acquiring skills in plant chromosome preparation techniques. Lastly, the third stage concentrated on refining skills to construct chromosome karyotyping. Completing all stages was required for participants, followed by submitting evaluation forms for assessment by the committee. The evaluation outcomes revealed that 84.30% of participants regarded the presentation and assistance provided by principal lecturer and assistants as highly commendable. At the same time, the remaining respondents expressed satisfaction with the quality and adequacy of the training. This comprehensive training effectively equipped participants with specialized chromosome and karyotype analysis skills, and the participants notably absorbed the training materials. The anticipated outcome of enhanced teacher understanding, specifically in the knowledge of inheritance and chromosome biology, was to stimulate heightened levels of student engagement and learning interest within the biology classroom.

1. INTRODUCTION

Biology, as a vast and continuously evolving field of science, encompasses a multitude of developing branches (Brooker et al., 2015). For educators, particularly biology teachers, the responsibility extends beyond the mere transmission of subject matter within the classroom setting; it necessitates the cultivation of proficient research data analysis skills specific to biology (Mertha et al., 2021). Furthermore, the role of biology teachers extends to the realm of pedagogical innovation, where they are tasked with the development and implementation of novel learning strategies to benefit their students (Carolina & Suryani, 2021). These innovations are instrumental in supporting the teaching and learning endeavors of school students (Sugiharto, 2013). It is imperative to underscore that inaccuracies in the interpretation and communication of biological theories can engender profound misunderstandings among students, underscoring the critical importance of equipping biology educators with the requisite analytical and pedagogical skills to foster a deeper and more accurate comprehension of this complex scientific discipline.

Biology educators must comprehensively understand genetics as it encompasses fundamental principles related to the inheritance of traits and the genetic material that governs

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these processes (Brooker et al., 2015). The genetic material in all organisms is composed of nucleic acids, specifically DNA or RNA (Clark & Donoghue, 2018). In eukaryotic organisms, this genetic material is intricately organized within a membrane-bound structure known as the nucleus (Efremov et al., 2022). As cells prepare for division, the linear and extensive genetic material undergoes a process of condensation, resulting in the formation of a highly dense substance visible under a light microscope. This condensed genetic material is called a chromosome (Billa et al., 2022). Chromosomes, when extended, are composed of DNA structures intricately bound to histone proteins (Paulson & Vagnarelli, 2011). This DNA-histone complex is further organized into a nucleosome strand (Díaz-Ingelmo et al., 2015), which, in turn, undergoes multiple twists and turns to yield the compact and intricate structure characteristic of a fully formed chromosome (Zhang et al., 2022). A profound grasp of these genetic intricacies is imperative for biology teachers to impart genetics principles to their students effectively.

Eukaryotic organisms exhibit varying chromosome numbers that continue to evolve (Clark & Donoghue, 2018; Guerra, 2008; Mayrose & Lysak, 2021). During the cell division phase, the count of chromosomes becomes discernible (Aristya & Alyza, 2019). This count can then be systematically arranged based on the ratio between the total arms and short arm length, yielding a chromosomal karyotype (de Resende, 2017). Chromosomal karyotyping holds significant utility, with applications ranging from identifying genetic abnormalities in individuals to investigating evolutionary processes and cytogenetic determination of sex, even enabling sex prediction (Bickmore, 2001; de Resende, 2017; Jackson, 1971). The practice of chromosomal karyotyping thus plays a pivotal role in various fields of genetics, providing valuable insights and applications that contribute to our understanding of genetics and its implications in diverse biological contexts.

Generally, most biology teachers in Samarinda study the theory of inheritance primarily through books and other written sources, which limits their in-depth understanding of the theory's implications. Hands-on learning can address this issue effectively. Providing theoretical knowledge complemented by practical experience, such as preparing samples, can significantly enhance understanding and leave a lasting impression.

To enhance the foundational grasp of genetics among biology educators, it is imperative to provide specialized training in chromosome and karyotype analysis techniques (Mertha et al., 2020). This targeted training endeavor aims to elevate biology teachers' comprehension of the intricate realm of inheritance. The premise underlying this effort is that an augmented understanding on the part of educators will invariably translate into heightened learning engagement among their students. This positive correlation arises from the fact that the delivery of genetic concepts and material becomes considerably more effective as teacher comprehension and proficiency increase, fostering an environment conducive to more profound and meaningful learning experiences in genetics.

2. METHOD

The community service initiative was carried out by a Chromosome and Plant Karyotype Analysis Techniques Training Team, which included lecturers, educational staff, and students from the Undergraduate Program of Biology in the Department of Biology, Faculty of Mathematics and Natural Sciences, at Mulawarman University. The training occurred on August 12, 2023. It served as a professional development platform for 21 biology educators from high schools, MAs, and equivalent educational institutions in Samarinda and its neighboring regions, as outlined in Attachment 1. Before the event, the team sent a request letter to the relevant schools, asking them to send biology teachers willing to attend the training. Teachers with a background in the field and laboratory experience in genetics were prioritized for selection.

The training program unfolded in a structured manner, comprising three distinct stages. In the initial stage, the participants were immersed in essential knowledge of inheritance fundamentals, basic chromosome analysis techniques, and plant chromosome karyotype analysis techniques. This phase was facilitated by Muhammad Fauzi Arif, M.Sc., serving as the principal lecturer. Subsequently, in the second stage, the participants engaged in hands-on training, gaining practical experience in plant chromosome preparations. This phase was conducted under the principal lecturer's direct guidance, bolstered by the assistant's support. In the culminating third stage, participants further honed their skills through practice in constructing plant chromosome karyotypes. This stage was facilitated by the principal lecturer, again in tandem with the assistance of the assistant. This carefully structured training program aimed to equip participants with a comprehensive understanding and proficiency in chromosome and karyotype analysis techniques, ensuring a well-rounded educational experience.

Participants were instructed in the techniques for the direct preparation of plant chromosomes. The protocol followed was adapted from Aristya & Alyza (2019) with certain modifications. Initially, shallot root tips were carefully excised between 08:00 and 10:00 am and promptly fixed in 45% glacial acetic acid for 15 minutes at a temperature of 4 °C. The fixed root tips underwent a thorough rinsing of three cycles with distilled water. The samples were then subjected to maceration using 1N HCl for 7 minutes at 60 °C. Post-maceration, the samples were rinsed thrice with distilled water. To facilitate visualization, the samples were stained with a 4% acetoorcein solution at room temperature for 120 minutes. Subsequently, root tips exhibiting enhanced staining intensity were selected and trimmed. These selected root pieces, enriched in coloration, were then treated with glycerin and covered with a glass coverslip. Gentle pressure (squash technique) was applied to disperse the cellular content evenly onto the coverslip. Finally, the prepared slides were sealed with acetone and examined under a light microscope. Several obstacles were encountered such as difficulty counting the number of chromosomes, difficulty finding the microscope's focal point due to participants' unfamiliarity with microscopes, and issues with installing software on participants' devices with different operating systems.

3. RESULT AND DISCUSSION

3.1 Delivering the basics of inheritance, chromosome analysis, and karyotype analysis of plant chromosomes

In the training program, the initial step entailed the provision of foundational instructional material to the participants. This step was crucial to ensure that participants could have a basic understanding of the subject matter, particularly if they have not previously been exposed to it. For those participants who had encountered this material in prior studies, this step served as a valuable refresher to reinforce their knowledge. This fundamental material was disseminated in the Theater Room, Science Learning Center, Faculty of Mathematics and Natural Sciences at Mulawarman University. This strategic choice of venue facilitated effective knowledge transfer and engagement among the training participants.

Preceding the dissemination of instructional material, participants were administered a pretest as a preliminary assessment tool. The primary purpose of this pretest was to gauge the participants' initial level of comprehension regarding the fundamental material that would subsequently be presented. Additionally, the principal lecturer employed the results of these pretests to effectively tailor the pace and depth of the material delivery to suit the participants' needs. The pretest administration was conducted online via the link `https://s.id/pretestkromosom2023`, enabling participants to access and complete it from their devices, as depicted in Figure 1. This pretest comprised five questions, each to be completed within 10 minutes, as illustrated in Figure 2.

Figure 1 . Training participants take a pretest on their respective devices

Analysis of the pretest outcomes revealed that most participants exhibited a commendable foundational grasp of biology, as evidenced by their responses aligning with the theoretical concepts being assessed (Table 1). Conversely, the pretest findings indicated that the vast majority of participants had not previously engaged in the direct preparation of chromosomes, with a mere 12% reporting prior experience in this regard (Figure 3). Notably, this training session served as the inaugural exposure to chromosome preparation techniques for the remaining 88% of the participants, emphasizing the novelty and importance of this training intervention in equipping biology educators with essential practical skills in chromosome analysis.

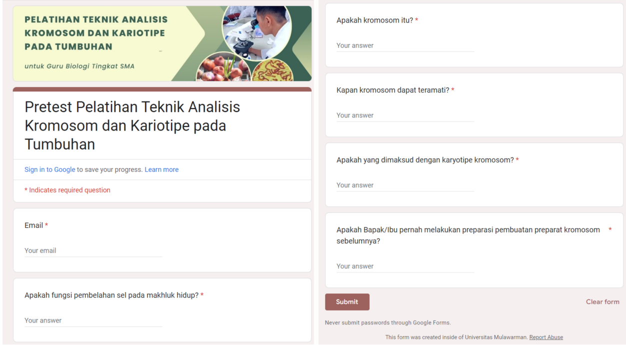

Figure 2 . Screenshot of pretest questions from `https://s.id/pretestkromosom2023`

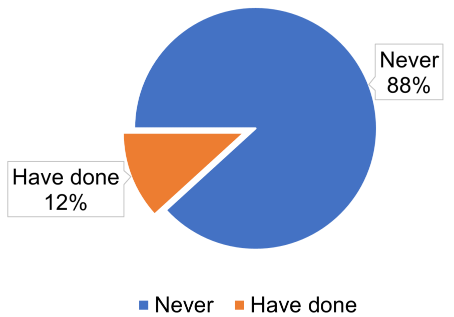

Figure 3 . Percentage of participants who have carried out chromosome preparations

The foundational material encompassed several critical facets of inheritance, including the comprehension of chromosomes, the intricate process of packaging genetic material into chromosomes, the dynamics of cell division, insights into the cell cycle, and a detailed exploration of the various phases of mitosis when cells undergo division. Furthermore, the training encompassed techniques for creating and arranging karyotypes on plant chromosomes. Additionally, it also included an examination of polyploidization in plants and its consequential impacts. After the presentation, a dedicated question and answer session allowed participants to engage with the principal lecturer. This interactive segment facilitated participant queries, several of which are documented in Table 2. The principal lecturer adeptly addressed participants' questions, ensuring clarity and comprehension. Following the resolution of all participant queries, the training program seamlessly transitioned into its subsequent stage, advancing to the practical hands-on activities of the second stage.

Table 1 . Answers to one of the pretest questions randomly taken from participants

Participants	Participants' answers to pretest question "What is a chromosome?"
Participant 4	Chromosome: molecule containing an organism's DNA
Participant 5	A collection of genes/DNA that determines the phenotype/genotype of living things
Participant 7	DNA molecule that contains the genetic material of an organism
Participant 11	A long DNA molecule that contains some or all of an organism's genetic material
Participant 13	DNA molecules containing genetic material

Table 2 . Questions from participants

Participants	Questions
Participant 8	What is the function of satellites in DNA? How does the structure look like?
Participant 11	Why the number of chromosomes in organisms vary?
Participant 8	Why do telomere sections on chromosomes always shorten?
Participant 12	Are there any bananas with seeds on the island of Borneo with diploid chromosome?
Participant 13	What is the technique for making plant chromosome preparations if the mitotic time remains unknown?

3.2 Practical techniques for plant chromosome preparations directly

During the second stage of the training program, participants engaged in practical exercises focused on the direct preparation of plant chromosomes under the guidance of assistants. These chromosome preparations were executed at the Laboratory of Animal Anatomy and Microtechnique, Faculty of Mathematics and Natural Sciences, Mulawarman University. To facilitate effective instruction and individualized attention, participants were organized into five distinct groups, each comprising four or five participants, accompanied by an assistant. Within this session, participants were systematically trained in the intricacies of producing plant chromosome preparations, aiming to enhance their proficiency and comprehension of these specialized techniques.

Allium cepa L., commonly known as shallots, was selected as the plant species of choice for preparing chromosomes in this training. The choice of shallots was motivated by several factors, including their accessibility, a relatively modest number of chromosomes, and the relatively large size of their chromosomes, as documented in previous research (Nefic et al., 2013). The selection of organisms with a reduced chromosome count and larger chromosome sizes is deliberate, as it simplifies the process of chromosome counting and analysis for educational purposes, facilitating a more comprehensible learning experience for participants.

The preparations began with cutting the root tip about 0.5 to 1 cm at 08.00 to 10.00 in the morning. Cutting was done according to the mitotic time. Root cutting must be timely because each organism has a different mitotic time (Annisa & Widodo, 2021; Kumar & Thonger, 2016). The chromosomes can be observed when the cell enters its mitotic time. After the roots were cut, the cuts were immediately fixed using 45% glacial acetic acid. The purpose of fixation is to stop cell activity after cutting (Daryono et al., 2013). For maceration, 1 N HCl was used. HCl was used to lyse the middle lamella because the lamella blocks the cells during microscope observation and made the squashing process difficult (Preeda et al., 2007). The staining agent used was aceto orcein 4%. This agent is perfect for staining several plant cell chromosomes (Olorunfemi et al, 2012). The roots were squashed to flatten the treated cells, and the chromosomes can be observed.

During this training stage, each participant undertakes the preparation process with the guidance of a dedicated assistant, as depicted in Figure 4. The role of the assistant encompasses elucidating the intricacies of the preparation techniques and addressing any inquiries raised by the participants. Participants had the opportunity to pose questions to the assistants within their respective groups, facilitating clarification and comprehension.

Figure 4 . Participants making plant chromosome preparations accompanied by the assistant

Preparations successfully crafted by participants were subsequently subjected to direct observation under a light microscope, employing a magnification of 10 × 40. In instances where chromosomes were discernible within the preparations, these findings were meticulously documented utilizing either an Optilab or a digital camera. With the successful completion of this stage, the training program seamlessly transitions into its final phase, ensuring participants acquire a comprehensive and practical understanding of the techniques for plant chromosome preparations.

3.3 Practical techniques for making plant karyotypes

The final phase of the training program was dedicated to providing participants with practical expertise in creating chromosome karyotypes. Under the direct guidance of the principal lecturer, participants embarked on hands-on training to construct karyotypes using previously documented preparation images. The initial step involved employing Image Raster version 3 software to measure the lengths of chromosome arms. Subsequently, participants calculated the centromere index for each chromosome, a crucial step in chromosome sequencing. The process entails arranging chromosomes based on their centromere index values, with the arrangement progressing from chromosomes possessing a higher centromere index to those with a lower index. These karyotypes were prepared and organized by utilizing Corel Draw version 23 software, allowing participants to gain practical experience in this essential aspect of chromosome analysis.

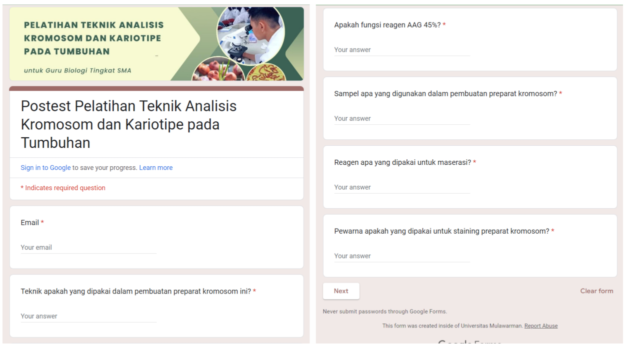

Figure 5 . Screenshot of posttest questions from `https://s.id/postestkromosom2023`

Following the comprehensive explanation provided to participants regarding the technique for constructing plant chromosome karyotypes, a crucial assessment step was implemented as a posttest. The primary objective of this posttest was to evaluate the extent to which participants had effectively assimilated the training material. The posttest was conducted online through the designated link `https://s.id/postestkromosom2023` (Figure 5), with participants tasked with completing the test within 10 minutes using their electronic devices. The posttest consisted of five essay questions to gauge participants' comprehension of the training content. Notably, the results of the posttest revealed that all participants demonstrated a commendable grasp of the training material, with nearly all questions answered accurately, underscoring the effectiveness of the training program in enhancing participants' understanding and proficiency in the realm of plant chromosome karyotypes.

3.4 Training evaluation by participants

The training program culminated with participants completing an evaluation sheet, a requisite task for each participant, designed to solicit feedback for the committee's appraisal. The evaluation encompassed multiple components, including the performance of principal lecturer, the quality of instructional materials, and the adequacy of facilities and infrastructure, as delineated in Table 3. The evaluation outcomes revealed that a notable majority, comprising 84.30% of participants, regarded the explanations provided by the principal lecturer as excellent, attesting to the effectiveness of the instructional delivery. The remaining participants acknowledged the quality of the explanations as excellent and sufficient. Similarly, 84.30% of participants expressed high satisfaction with the teaching and assistance rendered by the assistants, deeming it excellent, while the remaining participants found the support provided to be reasonable and adequate. Moreover,

Table 3 . Results of participants' evaluation of the training

No.	Description	Assessment Scale (%)				
		5 (Very good)	4 (Good)	3 (Enough)	2 (Not good)	1 (Not very good)
1	What is your response to the principal lecturer's presentation of material?	84,30%	10,50%	5,20%	0,00%	0,00%
2	What is your response to the training assistants of material?	84,30%	10,50%	5,20%	0,00%	0,00%
3	What is your response to the training implementation process?	84,30%	10,50%	5,20%	0,00%	0,00%
4	What is your response to the facilities and infrastructure supporting the implementation of the training?	68,41%	31,60%	0,00%	0,00%	0,00%
5	What is your response to the facilities and infrastructure supporting the implementation of practicums in the laboratory?	68,41%	31,60%	0,00%	0,00%	0,00%

the practical implementation of the training program received overwhelmingly positive assessments, with 84.30% of participants rating it as very good, signifying the practical utility and effectiveness of the hands-on components of the training program.

A noteworthy concern that remains prominent for the committee pertains to the adequacy of facilities and infrastructure essential for successfully executing training and laboratory practicums. This concern is substantiated by the feedback received from participants, wherein merely 68.41% of respondents rated the facilities and infrastructure as excellent, with a significant proportion, amounting to 31.60%, indicating a good rating. These findings underscore the imperative for the committee to enhance and optimize the supporting facilities and infrastructure in anticipation of future training endeavors. Furthermore, participants proactively contributed constructive suggestions for improving forthcoming training initiatives, highlighting their active engagement in continuously enhancing the educational experience.

The results of the pretest, materials, hands-on training, and posttest have indicated that after the training, participants had gained a deeper understanding of the material on the inheritance of traits, plant chromosome analysis, and karyotyping. This enhanced understanding is reflected in the participants' posttest responses, which show a greater focus on substance rather than memorization than pretest responses (the results are available and can be accessed through. This improved comprehension can be valuable for teachers when instructing on genetics-related subjects (the participants' results can be accessed via: http://s.id/hasilpretest2023 and http://s.id/hasilposttest2023).

4. CONCLUSION

The training program encompassed a comprehensive range of activities, including providing instructional material covering fundamental concepts related to inheritance, chromosome analysis, and the analysis of plant chromosome karyotypes. Participants were also actively engaged in practical exercises involving the creation of plant chromosome preparations and the proficient construction of plant chromosome karyotypes. The participants demonstrated a high receptivity, effectively absorbing the presented material. This heightened comprehension of the foundational principles about inheritance and chromosomes is expected to have a positive ripple effect, augmenting the participants' capacity to ignite and sustain the learning interest of the students they instruct. Consequently, the training program is poised to facilitate more effective and engaging biology education in the classroom.

ACKNOWLEDGMENT

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CONFLICT OF INTERESTS

The authors state that there is no conflict of interest.

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ATTACHMENT

Attachment 1. List of training participants and the school of each participant

No.	Participants	Affiliation	Affiliation Address	Course Subject
1	Participant 1	SMA Negeri 10 Samarinda	Jl. PM. Noor No.1, Sempaja Selatan, Samarinda Utara, Samarinda, Kalimantan Timur	Biology
2	Participant 2	SMA Negeri 10 Samarinda	Jl. PM. Noor No.1, Sempaja Selatan, Samarinda Utara, Samarinda, Kalimantan Timur	Biology
3	Participant 3	SMA Negeri 10 Samarinda	Jl. PM. Noor No.1, Sempaja Selatan, Samarinda Utara, Samarinda, Kalimantan Timur	Biology
4	Participant 4	SMA Negeri 3 Samarinda	Jl. Ir. H. Juanda No.20, Air Hitam, Samarinda Ulu, Samarinda, Kalimantan Timur	Biology
5	Participant 5	SMA Negeri 3 Samarinda	Jl. Ir. H. Juanda No.20, Air Hitam, Samarinda Ulu, Samarinda, Kalimantan Timur	Biology
6	Participant 6	SMA Negeri 3 Samarinda	Jl. Ir. H. Juanda No.20, Air Hitam, Samarinda Ulu, Samarinda, Kalimantan Timur	Biology
7	Participant 7	SMA Negeri 3 Samarinda	Jl. Ir. H. Juanda No.20, Air Hitam, Samarinda Ulu, Samarinda, Kalimantan Timur	Biology
8	Participant 8	SMA Negeri 14 Samarinda	Jl. Ringroad, Lok Bahu, Sungai Kunjang, Samarinda, Kalimantan Timur	Biology
9	Participant 9	SMA Negeri 10 Samarinda	Jl. PM. Noor No.1, Sempaja Selatan, Samarinda Utara, Kota Samarinda, Kalimantan Timur	Biology
10	Participant 10	SMA Negeri 4 Samarinda	Jl. KH. Harun Nafsi No.40, Rapak Dalam, Loa Janan Ilir, Samarinda, Kalimantan Timur	Biology
11	Participant 11	MA Sabilarrasyad Samarinda	Jl. Pusaka, Lok Bahu, Sungai Kunjang, Samarinda, Kalimantan Timur	Biology
12	Participant 12	MA Negeri 2 Kutai Kartanegara	Jl. Jelawat No.51, Timbau, Tenggarong, Kutai Kartanegara, Kalimantan Timur	Biology
13	Participant 13	SMA Negeri 3 Unggulan Tenggarong	Teluk Dalam, Tenggarong Seberang, Kutai Kartanegara, Kalimantan Timur	Biology
14	Participant 14	SMA Negeri 2 Muara Kaman	Benua Puhun, Muara Kaman, Kutai Kartanegara, Kalimantan Timur	Biology
15	Participant 15	SMA Negeri 1 Tenggarong	Jl. Mulawarman No.31, Sukarame, Tenggarong, Kutai Kartanegera, Kalimantan Timur	Biology
16	Participant 16	SMA Negeri 1 Muara Badak	Jl. Gas Alam, Batu-Batu, Muara Badak, Kutai Kartanegara, Kalimantan Timur	Biology
17	Participant 17	SMA Negeri 1 Loa Kulu	Loh Sumber, Loa Kulu, Kutai Kartanegara, Kalimantan Timur	Biology
18	Participant 18	SMA Negeri 5 Samarinda	Jl. Ir. H. Juanda No.1, Air Putih, Samarinda Ulu, Samarinda, Kalimantan Timur	Biology
19	Participant 19	SMA Negeri 7 Samarinda	Jl. Soekarno Hatta, Tani Aman, Loa Janan Ilir, Samarinda, Kalimantan Timur	Biology
20	Participant 20	SMA Negeri 7 Samarinda	Jl. Soekarno Hatta, Tani Aman, Loa Janan Ilir, Samarinda, Kalimantan Timur	Biology
21	Participant 21	SMA Negeri 1 Tenggarong Seberang	Bukit Pariaman, Tenggarong Seberang, Kutai Kartanegara, Kalimantan Timur	Biology