Use of genetic similarity analysis for identification and study of the origin of Indonesian local goats based on X-Y sex chromosome karyotyping and cytochrome-b genes sequence

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Abstract. The history and origins of Indonesian local goats are still compiled and described based on information on phenotypic characters, and there is still limited research that studies the origin of goats based on cellular and molecular analysis. The purpose of this study was to identify the origin of local goats in Indonesia based on the emergence of the profile of the sex chromosome band and the results of the Cytochrome b (Cyt-b) gene sequence. The research method is a case study with analysis of sex chromosome band profile results of preparation with G-banding and Cyt-B gene sequences. Genetic similarity analysis was performed using mitochondrial DNA (mtDNA). The sample used was whole blood from six Senduro goats, three male Etawah Grade and three Etawah goats. Chromosome preparation was done by G-Staining and analyzed by cytovision then analyzed its similarity with ancestor breeds. The whole blood sample was isolated with the Blood DNA Preparation Kit by Jena Bioscience. The primers used are Forward (Cytb_F) 5’GCAATTGCCATAGTCCACCT’3 and Reverse (Cytb_R) 5’GGATTGCC GGGTATAGTGT’3. The PCR results were sequenced by the Sanger method. Gene sequence analysis is performed using MEGA-X software. The results showed that the genetic distance between intraspecies and interspecies of Senduro goats and PE goats was 0% or ≤ 2%. Conclusions based on genetic similarity from this study are Senduro goats and Etawah Peranakan goats, and Etawah has a close kinship, instead there is a distant kinship with the comparison of Capra Hircus, but it has a close kinship between individuals.

1. Introduction
The history and origins of local Indonesian goats are still compiled and described mainly based on information on phenotypic characters. Most of the livestock animals in Indonesia are categorized as a non identified breed and practically have no sufficient genetic quality information, pedigree, and limited recording. There is still limited research that studies the origin of goats based on cellular and molecular analysis. Genetic analysis at the molecular and cellular level needs to be done, one of which is through chromosome analysis (sex chromosome) and Cytochrome-B gene analysis, which can reportedly be used as devices for identification and tracking of animal origins [1].
Indonesia is a country with high animal biodiversity, one of which is ruminant livestock, namely goats. Physical characteristics in Senduro goats are relatively similar to Etawa Peranakan goats because the Senduro goat is selected from PE goats by breeders and breeders for many years [2]. Both of these breeds are included in the genetic wealth of local Indonesian livestock. It is essential to preserve genetic resources to maintain and improve the quality of phenotypic and genotyping continuously so that both breeds can survive [3].

The Senduro goat is a breed derived from Etawa goats, wherein about 1947, Etawa goats were brought home to Java for development purposes through crossbreeding with local goats. Initially, the Senduro goat was only in the Senduro sub-district of Lumajang district located on the slopes of Mount Semeru, East Java Province [4]. Etawa Grade (PE) and Senduro goats are now widespread in goat production centers in several provinces in Indonesia. Diversity and genetic kinship can be used as a starting point to improve the quality and quantity of animals or individuals. Therefore, information about genetic crosses and genetic variation in Senduro and PE goats is crucial in the effort to breed these crossing goats to obtain superior animals. The purpose of this study was to identify the origin of local goats in Indonesia based on the emergence of the profile of the sex chromosome (X and Y) band and the results of the Cytochrome b (Cyt-b) gene sequence.

2. Experimental details
The research method is a case study with an analysis of sex chromosome profile results of preparation with G-banding and Cyt-B gene sequences. This study used nine male Senduro and PE goats, which are currently used as Artificial Insemination males for the production of frozen semen.

Genetic similarity analysis was performed using mitochondrial DNA (mtDNA). The sample used was whole blood from six Senduro goats, three male Etawah Grade, and three Etawah goats. Chromosome preparation was done by G-Staining and analyzed by cytovision then analyzed its similarity with ancestor breeds. The whole blood sample was isolated from the Blood DNA Preparation Kit by Jena Bioscience. The primers used are Forward (Cytb-F) 5’GCAATTGCCATAGTCCACCT’3 and Reverse (Cytb-R) 5’GGATTTTGCCGGGTATAGTT’3. The PCR results were sequenced by the Sanger method. Gene sequence analysis is performed using MEGA-X software.

3. Result and Discussion
3.1. Gene sequence analysis
The results showed that the genetic distance between intraspecies and interspecies of Senduro goats and PE goats was 0% or ≤ 2%. The genetic distance between Senduro goats intraspecies has a range of 0% - 2%, and the genetic distance between PE goats has a range of 0% - 1%, while the genetic distance between Senduro and PE goats interspecies ranges from 0% or ≤2%. Based on intraspecies genetic distance analysis, Senduro and PE goat interspecies have a very close kinship-genetic relationship level. This is because the Senduro and PE goats are goats that come from one species that are fellow but have different breeds.

The results of phylogenetic tree reconstruction with Neighbor-Joining Tree analysis show that Senduro goats and PE goats will be grouped into two different branches at a genetic distance of 0.002. (Figure 1). Based on genetic similarity from this study are Senduro goats and Etawah Peranakan goats. Etawah has a close kinship; instead, there is a distant kinship with the comparison of Capra Hircus, but it has a close kinship between individuals.

Based on intraspecies genetic distance analysis, Senduro and PE goat interspecies have a very close kinship-genetic relationship level. The results of phylogenetic tree reconstruction with Neighbor-Joining Tree analysis show that Senduro goats and PE goats will be grouped into two different branches at a genetic distance of 0.002. The genetic distance between Senduro goats intraspecies has a range of 0% - 2%, and the genetic distance between PE goats has a range of 0% - 1%, while the genetic distance between Senduro and PE goats interspecies ranges from 0% or ≤2%. Based on intraspecies genetic distance analysis, Senduro and PE goat interspecies have a very close kinship-genetic relationship level. This is because the Senduro and PE goats are goats that come from one species that are fellow but have different breeds. The results of phylogenetic tree reconstruction with Neighbor-Joining Tree analysis show that Senduro goats and PE goats will be grouped into two different branches at a genetic distance of 0.002. [5].
3.2. Chromosome analysis of X-Y appeal intensity

The results showed that the genetic distance between intraspecies and interspecies of Senduro goats and PE goats was 0% or ≤ 2%.

Figure 1. A. Filogeneic tree and od two main local goat of senduro and PE compare to a several breeds of Capra Hircus [5]

Figure 2. A Comparation of Sex Chromosome intensity of G banding of Local Indonesian Goat: X chromosome (A) and Y chromosome (B). [6]
Patterns of X and Y chromosome-based intensity can be distinguished based on the peak intensity of more appeal [7]. In general, the X chromosome shows more peak intensity, as much as 4 - peak band intensity. In contrast, the Y chromosome is 1-2 peak bands. Further research needs to be further studied in specific prophetic profile of chromosome band intensity which can be used as a basis for analyzing the similarity of goat individuals from different breeds, based on the comparative chromosome profile. Putri et al [6], have done chromosome analysis of PE and Senduro goats, and found that the X-Y chromosomes of the two goat breeds did not differ based on the morphological shape of the chromosomes.

Conclusions based on genetic similarity from this study are Senduro goats and Etawah Peranakan goats, and Etawah has a close kinship. Instead, there is a distant kinship with the comparison of Capra Hircus, but it has a close kinship between individuals. Sex Chromosome Analysis can be used to identify Breed (Similarity)

4. Conclusion
This study aimed to identify the historical origin of local goats in Indonesia based on the emergence of the profile of the sex chromosome band and the results of the Cytochrome b (Cyt-b) gene sequence. The research method is a case study with a descriptive analysis of the sex chromosome band profile of preparation with G-banding and Cyt-B gene sequences. Genetic similarity analysis was performed using mitochondrial DNA (mtDNA). The sample used was whole blood from six Senduro goats, three male Etawah Peranakan, and three Etawah goats. Chromosome preparation was done by G-Staining and analyzed by cytovision then analyzed its similarity with the Blood DNA Preparation Kit by Jena Bioscience. The primers used are Forward 5GCAATTGCGATGTCACCT3 and Reverse 5GGATTGGCC GGGGTATAGT3. The PCR results were sequenced by the Sanger method. Gene sequence analysis is performed using MEGA-X software. The results showed that the genetic distance between intraspecies and interspecies of Senduro goats and PE goats was 0% or ≤ 2%. Conclusions based on genetic similarity from this study are Senduro goats and Etawah Peranakan goats, and Etawah has a close kinship. Instead, there is a distant kinship with the comparison of Capra Hircus, but it has a close kinship between individuals.

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