Relatedness and diversity in Swedish local chicken breeds using mitochondrial DNA D-loop sequences and SNP data

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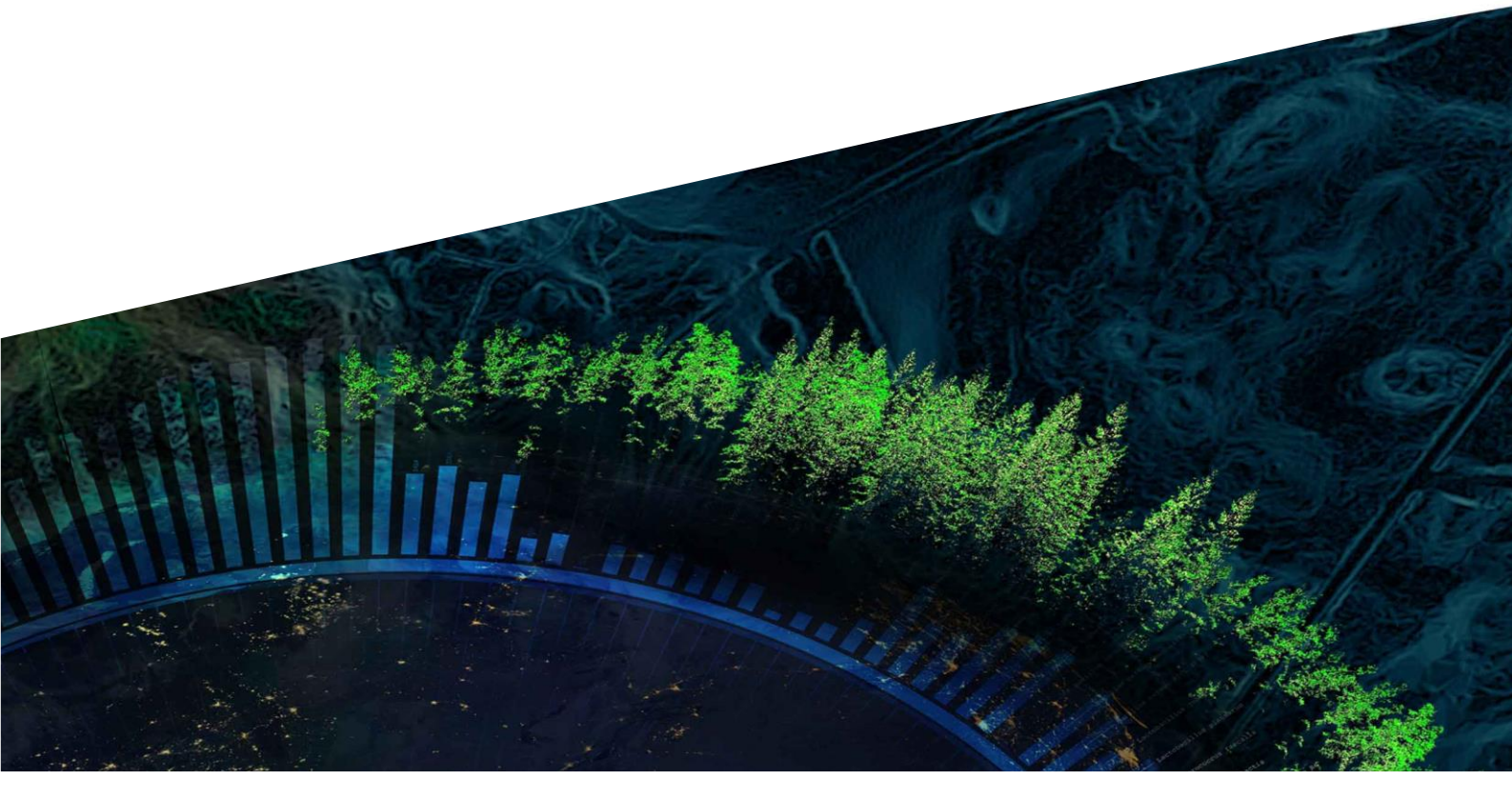

RELATEDNESS AND DIVERSITY IN SWEDISH LOCAL CHICKEN BREEDS USING MITOCHONDRION DNA D-LOOP SEQUENCES AND SNP DATA

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Abstract

Genetic diversity is the variation in genetic information carried by individuals within a species or population. This variability plays a crucial role in enabling species to adapt and survive in changing environments and is essential for evolutionary processes. Additionally, genetic diversity might carry genetic variants that improve resistance to diseases. In this study, a genetic analysis was carried out to investigate the genetic diversity, evolutionary history, and inter-breed relationships of Swedish local chicken populations. The initial 533 base pairs in the hypervariable D-loop region of the mitochondrial DNA was sequenced from 64 individuals representing 13 different breeds, and autosomal SNP data generated from 83 individuals representing eight different breeds was used. The study found that a total of 16 polymorphic sites and 9 distinct genetic sequences (haplotypes) were identified among the 13 Swedish local chicken breeds using the mtDNA data. Clade nomenclature in this study was according to the nomenclature of clades reported in previous studies, where domestic chickens were categorized into nine distinct clades (A to I) based on their association with areas of domestication and geographical dispersal. The majority of the breeds were found to belong to Clade E, while others belong to clades A and B, which suggests that the Swedish local chickens have several maternal lineages that likely originated from the Indian subcontinent, Yunnan province, and the regions of China. The analysis of molecular variance (AMOVA) revealed a higher degree of genetic variation between different chicken populations than within the same population. The nucleotide diversity of the studied breeds was found to range from 0 to 0.0169. An analysis of the mitochondrial DNA D-loop data using a neighbor-joining phylogenetic tree indicated that the Swedish indigenous chicken breeds share a closer evolutionary relationship with haplotypes present in European chicken breeds, rather than those found in Asian breeds. The principal component analysis of the SNP data revealed a closer genetic relationship among five of the Swedish local chicken breeds (Ölandshöna, Öländsk dvärghöna, Skånsk blommehöna, Äsbohöna, Kindahöna), while the other three breeds (Gotlandshöna, Hedemorahöna, and Svarthöna) were observed to be distinct. This research contributes to the understanding of genetic variation in the indigenous populations, and the findings may be applied to develop appropriate breeding and conservation strategies for the Swedish local chicken breeds. For a more comprehensive understanding of genetic diversity, origin, and relationships, it is recommended to perform analyses using both mitochondrial and nuclear DNA.

Keywords: chicken, genetic diversity, mtDNA d-loop, SNP, Swedish local chicken breeds

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Abbreviations

Asbo	Äsbohöna
Bjur	Bjurholmshöns
Bp	base pairs
Chr	Chromosome
Dalsk	Dalsk pärlhöna
D-loop	Displacement loop
DNA	Deoxyribonucleic acid
F	Inbreeding coefficient
F_{IS}	Individual heterozygosity relative to subpopulation
F_{ST}	Fixation index
Gammel	Gammelsvensk
Geno	Missing genotype rate
Gotl	Gotlandshöna
He	Expected heterozygosity
Hedemo	Hedemorahöna
Ho	Observed heterozygosity
Kinda	Kindahöna
MAF	Minor allele frequency
Mind	Missing genotype rate
mtDNA	Mitochondrion DNA
Öland	Ölandhöna
ÖlDvarg	Öländsk dvärghöna
Orust	Orusthöna
PCA	Principal component analysis
PIC	Polymorphic information content
Skblom	Skånsk blommehöna
SNP	Single nucleotide polymorphic
Svart	Svarthöna
Torpard	Torpardvärghöna

1. INTRODUCTION

1.1 Background

One of the most frequently produced livestock species on the planet is the domesticated chicken (*Gallus gallus domesticus*), as the chicken sector provides an efficient means of supplying the growing world population with the essential animal protein. The chicken species has undergone extensive diversification through the predominant evolutionary mechanisms of genetic mutation, artificial selection, adaptation, reproductive isolation, and random genetic drift. Over the course of multiple generations, this has resulted in the emergence of numerous breeds and strains, each with unique traits and differing levels of performance. The implementation and prioritization of advanced selection programs have facilitated the enhancement of genetic traits in various breeds, ultimately leading to the substitution of local breeds with more productive ones on a global scale. The local breeds were at risk of going extinct as improved varieties like the White leghorn gained massive superiority in performance, which led to a growing concern about genetic resources being eroded (FAO, 2015). These local chicken breeds have a high genetic diversity that can increase the probability of survival in different environments and conditions (Granevitze et al., 2007; Islam and Nishibori, 2009). The preservation of local breeds is deemed essential for ensuring future breeding possibilities as their genetic diversity is likely to play a crucial role in the development of current or future traits of significance, as stated by Bruford et al. (2003) and Toro et al. (2008).

Swedish local chicken breeds originating from various regions of Sweden faced the threat of extinction and experienced a severe bottleneck. However, some of these breeds were preserved and became rare, yet still exhibit significant phenotypic variability (Johansson and Nelson, 2015). It is therefore crucial to extensively identify and assess genetic resources to establish effective programs for their utilization and protection. Maintaining genetic variation in farm animals can be achieved through the preservation of variety of breeds and by using genetic technologies and techniques such as cryoconservation, invitro fertilization, gene editing, genotyping (Soller et al., 2006).

Mitochondrial DNA (mtDNA) is a type of DNA that is found in the mitochondria of cells and is inherited maternally. It has become a commonly utilized marker in molecular research, due to its simple molecular structure and rapid evolution (Lan et al., 2015). In accordance with the methods used for other animal species, the utilization of mitochondrial DNA sequences has been implemented to investigate the evolutionary relationships between various poultry species through scientific inquiry. Nishibori et al. (2005) used the whole mitochondrial DNA sequencing and a partial nuclear genome analysis to identify potential hybridization and interbreeding events among different species of the genus Gallus, including the gray jungle fowl, red jungle fowl, domestic chickens, and Ceylon jungle fowl. Multiple studies have been carried out using mitochondrial genome (mtDNA) sequencing to investigate the genetic diversity and relationships among wild chickens, local breeds, and commercially reared chickens (Nishibori et al., 2004, 2005).

The starting site for mitochondrial DNA replication is in the D-loop region (regulatory region), and the entire mtDNA D-loop sequence provides more information and unbiased data essential for characterizing, conserving, and promoting invaluable genetic resources (Islam et al., 2019). The D-loop region is a non-coding region of the mtDNA that has been extensively employed in population genetics studies due to its high level of polymorphism. According to Muchadeyi et al. (2008), the rate of evolution in the D-loop region of mtDNA is five to ten times greater than that observed in other regions, which may be due to the D-loop region's crucial role in mtDNA replication, serving as the site of initiation, combined with the high mutation rate and the limited repair mechanisms, and this has led to the utilization of mtDNA D-loop in studies related to genetic diversity and phylognetic analysis, and has been extensively applied in comprehending phylogenetic relationships in various animal species such as pig, cattle, sheep, goat, and chicken (Giuffra et al., 2000; Bhuiyan et al., 2007; Hiendleder et al., 2002; Chen et al., 2005; Liu et al., 2006). Through studies of the maternally inherited mtDNA D-loop region, it is possible to assess the genetic diversity, ancestry, and evolutionary history of the chicken genetic resources (Osman et al., 2016; Nishibori et al., 2004).

A SNP is a genetic variation that involves the replacement of one nucleotide with another at a specific location or position within the DNA sequence of its entire genome, it occurs and is present in a sizable proportion. SNPs are valuable tools as genetic markers for identifying genetic risk factors for diseases, investigating the inheritance of traits, and exploring the connection between genetic variation and phenotypic variation in both population genetics and medical research. Autosomal SNP data has been utilized to evaluate the magnitude of genetic variation in the populations, to identify those with high rates of inbreeding, and to assess the genetic relatedness of individuals within populations (Pritchard et al., 2000; Malomane et al., 2018). The DNA sequence have a high number of SNPs per unit area, which

results in a substantial amount of genetic information being collected thereby providing a more comprehensive understanding of population structure, gene flow, inbreeding, and individual relatedness.

This research utilized two sets of data, comprising the D-loop sequence data of thirteen Swedish local chicken mtDNA and SNP data of eight of the breeds for the evaluation of relatedness and genetic diversity. The breeds to be studied included, Dalsk pärlhöna, Gammelsvensk dvärghöna, Äsbohöna, Bjurholmshöns, Orusthöna, Kindahöna, Ölandshöna, Gotlandshöna, Öländsk dvärghöna, Hedemorahöna, Skånsk blommehöna, Torpardvärghöna, Svarthöna.

1.2 Statement of Problem

The degree of the genetic variation, relatedness and maternal origin of the local chicken population in Sweden is not well established due to limited information. Therefore, the objective of this scientific study is to evaluate the extent of genetic diversity, relatedness and the maternal origin of the Swedish local chicken breeds using mtDNA D-loop data and SNP data. This research will provide a greater understanding of the genetic composition of the local chicken population in Sweden and contribute to the preservation and sustainable use of these breeds. Conservation efforts can enhance the survival probability of these endangered breeds and preserving their genetic diversity can promote the adaptability of future populations to altered environmental conditions, such as those arising from climate change (FAO, 2019).

1.3 Objectives

1. To characterize the genetic variation in the Swedish local chicken breeds
2. To assess the relatedness between the Swedish local chicken breeds
3. To assess the maternal origin of the Swedish local chicken breeds

2. LITERATURE REVIEW

2.1 Genetic Diversity in Chicken

The purpose of a genetic diversity study is to comprehend and measure the degree of genetic variation within individuals and populations, generating insights into their genetic composition and evolutionary history. Breeders' most valuable resource is genetic diversity as it affects how sustainably we can meet human needs through the provision of food and other necessities (Fadhil et al., 2018). Early breed improvement, which involved selective breeding, did initially lead to an overall increase in genetic diversity within domestic animal populations. This is because different breeds were developed for specific characteristics, such as meat or milk production, and this resulted in the selection of different sets of genes which increased genetic diversity within the overall population. After World war, there was a shift towards the development of highly specialized breeds that were often selected for a single trait or set of traits, and as a result, genetic diversity within these breeds was reduced as specific sets of genes were selected for. Despite this reduction in genetic diversity, an increase in animal productivity has been shown to positively impact production economics.

However, the Food and Agriculture Organization of the United Nations (FAO) predicts a 70% increase in the worldwide demand for animal products by 2050, this increase in demand places significant pressure on the animal agriculture industry to improve productivity, while also addressing environmental and social sustainability challenges. Research has shown that environmental factors, including climate change and changes in land use, have significant impacts on animal productivity and health (Gerber et al., 2013). Maintaining genetic variation in animal populations is necessary for ensuring sustainability in the face of these challenges, as genetic diversity can may increase the probability of resistance to environmental and disease-related stresses (Mpenda et al., 2019). Therefore, while there is a need to improve productivity to meet increasing demand, it is also important to balance this with the need to maintain genetic diversity within animal populations.

The ancestral origins of domestic chickens have been a topic of scientific debate for centuries, however, the widely accepted theory being that their origins can be traced back to the red junglefowl as first suggested by Charles Darwin in 1868. He made this comparison based on the anatomy and output of different species within

the Gallus genus. Over time, the genetic variation among chickens has been influenced by various populations and types, comprising the red junglefowl, local and purebred varieties, small-scale food producers, commercial stocks, and specific lineages. These populations have contributed to the diversity of genetic resources in chicken, leading to the wide range of breeds and varieties available today. Genetic diversity is critical to the success of the chicken industry, as it provides a wide variety of traits and characteristics that can be used to improve productivity and adaptability in different environments.

According to studies on the genetic variation of different breeds of chicken conducted across the world, traditional unselected breeds and Jungle Fowl populations are both highly heterogeneous populations that make up a sizable share of the overall genetic variability (Groeneveld et al., 2010). Mercan and Okumus (2015) found that the local chicken population were somewhat more polymorphic than the commercial hybrid chicken population in Turkey. A comprehensive investigation was conducted by Malomane et al. (2019) using 3235 chickens of 162 populations from 32 countries, using SNP data, the study found that the chicken population clustered in eleven groups according to their geographical origin and agricultural history. Native European breeds, mostly standardized breeds, were found to have little genetic diversity (Granevitze et al., 2007), and it is likely caused by limited effective population size and assortative mating, while the indigenous populations originating from Asia and Africa demonstrated significant genetic diversity and lacked a uniform demographic pattern.

Individuals' ability to reproduce and survive can be impacted by genetic variation or its absence (Mukhopadhyay & Bhattacharjee, 2016). In order to select and conserve breeds of animals, understanding the variation in their genetic composition and how it is maintained is crucial. Thus, it is necessary to assess the present state of variation and make predictions about how it may change in the future.

2.2 Local Breeds of Chicken

The genetic variation of the early animal species was derived from the local genetic resources, which have endured for many millennia under both favorable and adverse environmental effects, they possess the capacity to adapt and flourish in changing environmental and climatic conditions (Fadhil et al., 2018). In the event of extinction of these breeds, their distinctive traits would also be eradicated, including genetic attributes that may not presently be recognized or acknowledged but could potentially be of significance in the future. By preserving the local animal breeds and their genetic resources, we can ensure that future generations will have

access to a wide range of genetic variation which can be used to develop new and improved animal breeds (Ertuğrul et al., 2000).

Different parts of Sweden are home to different breeds of Swedish chicken. A few Swedish chicken breeds survived when they were saved from extinction. Despite the potential existence of severe bottlenecks, these breeds nonetheless show a wide range of phenotypes, such as in terms of body size and plumage color. Recent molecular genetic research (Englund et al., 2014; Abebe et al., 2015; Johansson and Nelson, 2015) have provided insight into the relationships between native Swedish chicken breeds, their population dynamics, and their genetic diversity. The molecular genetic marker systems used in these experiments included SNPs, microsatellites, and mitochondrial DNA (mtDNA). Olandshöna was distinguished from eight other studied varieties by mtDNA analysis, which revealed that Swedish regional chicken breeds were of differing maternal origins (Englund et al., 2014). In addition, according to Abebe et al. (2015), 24 microsatellite markers were used to examine the genetic variability, dispersion of genetic variability within and between populations, and level of genetic relationship among five indigenous chicken breeds in Sweden. This revealed that genetic variation in Svarthöna was low, and the level of molecular co-ancestry that was analysed with the Molkin v3.0 software (Gutiérrez et al., 2005), was high compared to other Swedish local chicken breeds used in the study. This suggests that in the past, relatives may have mated, leading to inbreeding in this population, which, if not well managed, can have negative effects on the population. There must be effective management and conservation of the native Swedish chicken breeds at all costs. There will likely be issues related to inbreeding depression and threats to the diversity of these breeds if they are not properly handled.

2.3 Importance of Genetic Diversity

To proficiently manage livestock genetic resources, it is essential to have a thorough understanding of breed traits, including information on genetic variability, population size and structure, production condition, and geographic distribution present among and within different breeds of animals (Groeneveld et al., 2010).

The genetic variability present both among and within different breeds of animals is vital for their survival and ability to adapt to changing environments. It allows populations to respond to challenges such as disease outbreaks, climate change, and other environmental pressures. Populations with higher levels of genetic diversity are more likely to have individuals with genetic variations, tolerance to changing environmental conditions, or improved productivity (Bickford et al., 2013). The survival and growth of animal populations rely on the presence of substantial

genetic diversity (Mukhopadhyay and Bhattacharjee, 2016). The population growth rate is likely to decreased as a result of reduced fertility and survival of inbred individuals, leading to an increased risk of population extinction.

Genetic diversity is important for breeding programs, as it enables the creation of novel lineages with enhanced characteristics. it allows for the development of new strains with improved traits. The importance of genetic diversity in breeding programs lies in its ability to provide a pool of genetic variation that can be utilized to develop new strains with improved traits. On the contrary, low genetic diversity limits the potential for improvement and increases the risk of genetic disorders and inbreeding depression (reduced fertility and survival). Therefore, preserving genetic variability is essential for the success of breeding programs and the sustainability of the livestock production systems. In general, the susceptibility of a trait to artificial selection is inversely proportional to its genetic variability.

2.4 Measures of genetic variation

The level of diversity in a species, population, subspecies, or breed can be determined by various factors such as the presence of specific genes, polymorphic loci percentage, allelic diversity, observed and expected heterozygosity. The possibility that two randomly selected alleles at a single genetic site in an organism with two sets of chromosomes (diploid organism) are distinct from one another is known as expected heterozygosity (He) (Mukhopadhyay and Bhattacharjee, 2016) The observed heterozygosity, which is estimated by dividing the number heterozygotes, by the sum of the number of loci that is studied, may differ from the expected heterozygosity due to drift or non-random mating effects.

From pedigree information, genetic diversity can be calculated. When relatives are mated, their offspring may have duplicate copies of the two alleles at one particular site. The coefficient of inbreeding (F) is a measure of the probability that an individual has inherited two identical copies of a gene from a common ancestor. The inbreeding coefficients for individuals and populations is commonly used in genetic studies. The inbreeding coefficient is a measure of the degree of relatedness between an individual and its ancestors, and it is based on the extent of the pedigree analyzed. The deeper the pedigree, the greater the chance of detecting inbreeding. As a result, it is expected that some level of inbreeding will be present in the calculated coefficients. However, these measures can vary between populations and may not be directly comparable. This means that higher inbreeding values may simply reflect the extensive depth of the pedigree record and not necessarily indicate a higher degree of inbreeding within the population.

Genetic diversity and structure of populations can be described using F-statistics (Wright, 1950; Caballero, 2013), it can depict fixation (index) that comes from describing the differences or similarities between subpopulations and the entire population (F_{ST}), as well as the inbreeding coefficient (F) of an animal in relation to the entire population (F_{IT}) or in relation to the subpopulation (F_{IS}) (Wright, 1951). F-statistics is useful in quantifying the extent of Wahlund effect and stratification within populations, the concept of Wahlund effect refers to a phenomenon in which genetic diversity is reduced within subpopulations due to limited gene flow between those subpopulations, which can lead to a loss of heterozygosity at a given locus. Stratification can also lead to a decrease in heterozygosity, as alleles are no longer evenly distributed across the entire population.

Another measure is nucleotide diversity, it is computed by dividing the number of nucleotide differences between two sequences by the sum of their frequencies and presented as a percentage. In human populations, two individuals chosen at random vary by around one autosomal single nucleotide polymorphism (SNP) per kilobase, while in chicken populations, the range is from 4 to 5.5, and in sheep and cattle, it is between 2 to 2.5, 1.4 in Angus and 3.4 in Holstein breeds (International Chicken Map Consortium, 2004; Villa-Angulo et al., 2009).

The Analysis of Molecular Variance (AMOVA) is a statistical approach commonly employed in population genetics, which is rooted in the principles of analysis of variance (ANOVA). Its purpose is to assess the genetic variation within and between populations. Excoffier et al. (1992) introduced AMOVA as a more capable alternative to other population genetic techniques that had difficulty dealing with numerous loci. For instance, various studies have applied AMOVA to explore the genetic diversity and structure of chicken population (Boudali et al., 2020), and cattle (Margeta and Margeta, 2019).

Principal Component Analysis (PCA) is a widely employed statistical tool to study the genetic variability both between and within populations. One of the primary applications of PCA in animal population genetics is to evaluate the genetic similarities between different populations of animal species. Wang et al. (2017) utilized PCA to investigate the genetic variabilities and relationships between the different breeds of domestic goats in China. In another study, Zhang et al. (2018) used PCA to explore the genetic diversity and differentiation in conserved chicken populations.

The median-joining haplotype network (MJN) is a widely utilized technique in population genetics to visually represent relationships between genetic haplotypes. The method employs the median-joining algorithm, which creates connections between haplotypes that differ by one or more mutations with a series of median

vectors, resulting in a network graph that displays both the relationships between haplotypes and the frequency of each haplotype within a population. MJN has been applied in various population genetics studies, including those concerning the origin and evolution of species, the genetic diversity and structure of populations, and the identification of genetic markers linked with particular traits or diseases. Additionally, MJN has been utilized to examine the genetic diversity and population structure of various animal and plant species, such as cattle and chicken (Mburu et al., 2003; Cuc et al., 2010). One of the advantages of the MJN technique is its ability to represent intricate relationships between haplotypes and populations in a straightforward and easily understandable manner.

Phylogenetic analysis, an important tool used in population genetics to understand evolutionary relationships and genetic diversity between and within populations. Phylogenetic analysis has been widely used to infer evolutionary relationships between species and populations (Felsenstein, 2004) and to study structure of genetic diversity and structure within and between populations (Maddison, 1997; Nei and Kumar, 2000). The neighbor-joining method (Saitou and Nei, 1987) has been used to construct phylogenetic trees that represent the genetic distances between individuals or populations.

2.5 Assessment of genetic diversity using molecular tools

Several genomic techniques have been developed to estimate genetic diversity within and between breeds. The most widely used marker type include Single Nucleotide Polymorphism (SNP) and microsatellites. Restriction Fragment Length. Polymorphism (RFLP) and the Amplified Fragment Length Polymorphism (AFLP) are techniques which were earlier applied (Yaro et al., 2017). These methods have been applied to various animal species, including chickens, cattle, pigs, sheep and horses, to evaluate genetic diversity, the evolutionary relationships and structure of the population.

One of the main benefits of using genomic techniques to estimate genetic diversity is that they can provide a large amount of information on a large number of loci. This is particular useful for conservation and breeding programs, as it allows for the identification of endangered breeds and the selection of genetically diverse individuals for breeding. Moreover, the use of SNP arrays enables the detection of rare variants, the identification of loci associated with specific traits, comparisons between families. Microsatellites are DNA regions composed of repeating units of short nucleotides, typically ranging from one to six base pairs, with the number of repeats varying among individuals (Rafalski et al., 1996; Reddy et al., 2002).

Microsatellites are highly informative but may not be evenly distributed throughout the genome, leading to potential biases in estimates of genetic diversity, they are less numerous than SNPs, may have “Null-Alleles” which are alleles that do not get amplified during the process of PCR amplification (Dakins and Avise, 2004; Reece et al., 2004), are more time consuming and comparison between labs/studies is limited.

SNP genotyping has become a popular method for assessing genetic diversity, as it allows for the concurrent genotyping of thousands of markers. Several researchers have used this approach to examine the genetic variation between breeds of chicken. A study by (Zhang et al., 2020) used SNP data to show that wild ancestral population in China have high levels of genetic diversity and are distinct from commercial breeds. Another study by (Liu et al., 2021) found that Chinese local chicken breeds are more diverse than commercial breeds and have a close relationship with wild ancestor. SNP technology has made it possible to collect detailed information on rare breeds with unique genetic makeup (Yaro et al., 2017), also, a comprehensive study by Malomane et al. (2019) used High Density (HD) SNP array data in 174 chicken populations around the world to assess diversity. However, the identification of SNPs in non-model organisms is challenged by the high costs and technical difficulties associated with existing SNP genotyping methods (Brumfield, 2003; Seddon et al., 2005; Garvin et al., 2010). Additionally, SNPs can be affected by ascertainment bias, which occurs when the SNPs selected for genotyping arrays are not chosen at random. This bias can result in genetic statistics deviating systematically from the expected theoretical value, causing population genetic measures to be underestimated (Geibel et al., 2021). One technique used to address this bias is LD pruning, which involves removing markers that are highly correlated with each other and retaining a subset of markers that capture the majority of genetic variation in the dataset.

2.6 Genetic Diversity within and between breeds

Studies have found that within-breed genetic diversity can be influenced by a variety of factors, including the breed's size, structure, and history of genetic bottlenecks (Leroy, 2011). Between-breed genetic diversity can be influenced by factors such as the breed's geographical origin, and demographic history. According to the genetic isolation by distance hypothesis, a population become more genetically distinct, and the amount of genetic diversity may decrease when that population migrates farther away from the population from which it originally descended (Nie et al., 2019). This is due to a reduction in mating chances across groups, which in turn reduces the amount of genetic exchange that occurs between populations (Cavalli-Sforza et al., 1964). However, according to Malomane et al.

(2021), studying the theory of genetic isolation caused by distance or the migration of chicken populations from a particular area can be difficult due to discrepancies between the current physical location of a chicken population and its actual geographic origin following migration from its original population. This can pose a challenge in many chicken breeds, as the exact timing of their migration to their present location is often unknown. However, the correlation between genetic distance and genetic diversity in distinct functional areas, such as genes and SNP classes, may vary based on their functional significance and can also differ across various breeds (Malomane et al., 2021).

The total genetic diversity in a population is also shaped by events that are specific to that population, including mutations, natural selection which promotes adaptation to the prevailing environments, and human-mediated selective breeding practices in livestock production (Ramachandran et al., 2005). These population-specific factors, as well as genetic drift, contribute to the overall genetic variation observed within a population. The observed and expected variability in heterozygosity is influenced by evolutionary factors such as random fluctuations in allelic frequencies (Nei, 1978). A decrease in genetic diversity within a breed can be attributed to various factors such as a small effective population size, genetic drift, inbreeding, specific breeding practices, and the lack of efficient breeding methods (Dakin and Avise, 2004; Aubin-Horth et al., 2005).

Genetic variation within and across breeds should be considered in conservation efforts for species or breeds (Toro et al., 2008). Weitzman (1992) has established a framework for analyzing genetic distances that provides insight into between-breed diversity. The Weitzman diversity method can be employed to evaluate operational taxonomic units, which may represent species, breeds, or subspecies. The technique involves computing distances between these units, with certain characteristics such as positivity, symmetry, and zero distance between an element and itself. The primary objective is to examine diversity between the units, but the method does not account for diversity within the individual units. Phylogenetic analysis can be used to quantify and display between-breed genetic differences (Marsjan and Oldenbroek, 2007), while heterozygosity is used to define the genetic variability within a breed (Caballero, 2013). Using cluster analysis, the different subpopulations within a breed and across breeds can be established (Toro et al., 2008).

2.7 Mitochondrial DNA

Phylogenetic systematics and genetic diversity can be estimated using mitochondrial DNA (mtDNA). The benefits of using mtDNA over nuclear DNA

are numerous, including rapid evolution which provide a source of genetic variation, the lack of introns, a high copy number that makes it easier to obtain and amplify for analysis, and less recombination, making it useful for inferring evolutionary relationships and for reconstructing population histories (Ladoukakis et al., 2017; Park and Larsson, 2011), but the mtDNA is inherited only on the maternal side which is a limitation. Due to the increased susceptibility to oxidative damage, along with replication errors and a limited mtDNA repair mechanism the rate at which mutations occur in mitochondrial DNA (mtDNA) is greater than the corresponding rate in nuclear DNA (Lynch et al., 2006; Park and Larsson 2011).

The regulatory functions of mitochondrial DNA (mtDNA) replication and transcription are attributed to the D-loop region. It accounts for approximately 7% of the entire length of the mitochondrial genome (Gabriel, 2012). The region is said to be the most variable part of mtDNA and has gained attention as a potential genetic marker for distinguishing between varieties or species, particularly in relation to various chicken breeds (Innak et al., 2019; Shadel and Clayton 1997). MtDNA D-loop sequence is observed to be mostly homozygous, and this is because it is maternally inherited and usually the same copy is present in each cell (Parakatselaki and Ladoukakis, 2021). During the process of cellular replication, the mitotic machinery only copies the mtDNA present in the mitochondria of the mother cell. This means that any variations in the mtDNA sequence will be passed down to the daughter cells and will be present in all the cells of an individual, which is probably the reason why mutations in mtDNA tend to result in transitions more often than transversions. In rare cases, a small proportion of cells may harbor a second mtDNA type known as a "heteroplasmy" (Parakatselaki and Ladoukakis, 2021). Heteroplasmy can occur through mutation, replication errors, or mitotic recombination. However, recombination in the mtDNA is thought to be rare, with most studies reporting little or no evidence for recombination events (Avise, 2000), but some studies have reported evidence for homologous recombination in the mtDNA, particularly in animals such as birds and mammals (Piganeau et al., 2004).

The sequencing of the D-loop region is a multistep process that involves DNA isolation, PCR amplification of the D-loop with specific primers, sequencing, and subsequent data analysis. DNA can be isolated from cells using a variety of techniques, such as differential centrifugation, sucrose gradient centrifugation, or column-based purification methods. The DNA is then purified and concentrated for use in downstream applications. PCR amplification of the mtDNA D-loop region is achieved through the use of primers that are specifically designed to bind to and target the D-loop of the mtDNA. The amplification of the mtDNA D-loop region using PCR yields numerous copies that can subsequently undergo sequencing. Various sequencing techniques, including Sanger sequencing, can be employed to sequence the amplified mtDNA D-loop region. Following sequencing, the obtained

data is examined to detect any mutations present in the mtDNA D-loop region. This can be done using a variety of bioinformatics tools and techniques, such as sequence alignment and variant calling. In most studies that investigate the genetic diversity, maternal origin, and phylogenetic relationships of domestic chickens, a partial sequence of the hypervariable D-loop region is utilized (Muchadeyi et al., 2008; Liu et al., 2006).

3. MATERIAL AND METHODS

This study employed two data sets, which included the D-loop sequence data of thirteen Swedish local chicken mtDNA and the SNP data of eight of the breeds.

3.1 PCR Amplification, Purification, and Sequencing

The present investigation involved the sequencing of the hypervariable region I (HVR1) of the mitochondrial DNA D-loop in 24 animals across seven different chicken breeds, namely Gammelsvensk (N=1), Bjurholmshöns (N=2), Orusthöna (N=2), Kindahöna (N=6), Öländsk dvärghöna (N=2), Dalsk pärlhöna (N=7), and Torpardvärghöna (N=4). Additionally, 40 mtDNA D-loop sequences obtained from a previous study conducted by Englund et al. (2014) were incorporated into the analysis, encompassing nine breeds, namely Orusthöna (N=2), Kindahöna (N=3), Ölandshöna (N=3), Gotlandshöna (N=8), Öländsk dvärghöna (N=4), Hedemorahöna (N=6), Skånsk blommehöna (N=5), Äsbohöna (N=4), and Svarthöna (N=5).

The D-loop region of chicken mtDNA was amplified using PCR following the procedures outlined in the Applied Biosystems BigDye® Direct Cycle Sequencing Kit manual (see protocol document in https://www.thermofisher.com/order/catalog/product/4458687). The resultant amplicons were then purified, and finally, the sequence of the DNA was determined.

A polymerase chain reaction (PCR) was carried out using a specific primer pair (Forward: 5'-AGGACTACGGCTTGAAAAGC-3' and Reverse: 5'-ATGTGCCTGACCGAGGAACCAG-3') to target the mitochondrial DNA (mtDNA) D-loop region and amplify a 533 bp fragment. The PCR reaction was composed of 1.0 µL of genomic DNA with a concentration of 4 ng/µL, 1.5 µL of a mix of forward and reverse primers with a concentration of 0.8 µM, 5.0 µL of BigDye® Direct PCR master mix, and 2.5 µL of deionized water. The amplification reaction was carried out using a thermal cycler under the following conditions: the reaction mixture was initially denatured at 96°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 62°C for 45 seconds,

extension at 68°C for 45 seconds, and final extension at 72°C for 2 minutes. The reaction was then held at 4°C.

To perform cycle sequencing, the product of the initial PCR was mixed with 1.0 µL of BigDye® Direct M13 forward or reverse primers (Forward: 5'-TGTAAAACGACGGCCAGT-3' and Reverse: 5'-CAGGAAACAGCTATGACC-3') and 2 µL of BigDye® Direct Sequencing master mix. This mixture was then subjected to thermal cycling. The thermal cycler was set to 37°C for 15 minutes, 80°C for 2 minutes, and 96°C for 1 minute, followed by 25 cycles at 96°C for 10 seconds, 50°C for 5 seconds, 60°C for 4 minutes, and 72°C, before being held at 4°C. The PCR product was purified using a premix of 45 µL SAM™ and 10 µL XTerminator® solution. Finally, the samples were run in a capillary electrophoresis instrument (3500xL DNA Analyzer, Applied Biosystems).

3.2 SNP Data editing and filtering

The current study used SNP data genotyped by DNA LandMarks in Canada for the GWMAS Consortium (Groenen et al., 2011) using a 62K SNP chip which was produced by Illumina. This dataset comprised of 83 animals of eight different breeds (Gotlandshöna, Kindahöna, Öländsk dvärghöna, Hedemorahöna, Ölandshöna, Skånsk blommehöna, Äsbohöna, Svarthöna) and 53,313 SNPs from the 28 autosomal chromosomes. To ensure quality, PLINK 1.90 was used to filter the data for an animal call rate of ≥ 95%, a minor allele frequency of at least 1%, and an SNP call rate of ≥ 99%. This resulted in the removal of 3,195 SNPs due to missing genotype, 3,799 SNPs due to minor allele frequency, and no animal was removed due to missing genotype data, leaving 46,319 SNPs and 83 chickens.

The application of linkage disequilibrium (LD) based pruning was employed as a technique to mitigate the influence of ascertainment bias in diversity analysis that utilizes SNP data (Malomane et al., 2018). For this purpose, PLINK 1.90 was employed with the following parameters: --indep-pairwise 50 1 0.5, these correspond to the parameters of window size, window shift, and r2 threshold (which involves eliminating markers with a pairwise r2 value exceeding 0.5) in the context of SNP data pruning. The pruning process led to the removal of 17,726 SNPs, leaving 28,593 SNPs for further analysis.

3.3 Data Analysis

The nucleotide sequences of the mitochondrial DNA (mtDNA) D-loop region in chickens from this study were analyzed using Chromas 2.6.4, modified with Bioedit Sequence Alignment Editor 5.0.9, and aligned utilizing MEGA software. (Kumar et al., 2008). The sequences were then stored in Bioedit format, and MEGA software was used to identify and designate any identical sequences as a single haplotype.

Alignment of the partial mtDNA D-loop sequences from 64 Swedish local chicken to a reference sequence from the GenBank (NC_007235.1) was conducted. The nucleotide diversity, as well as the polymorphic sites, and the number of haplotypes were analyzed to determine diversity, and an Analysis of Molecular Variance (AMOVA) was executed utilizing ARLEQUIN v. 3.5 software (Excoffier and Lischer, 2010) to quantify maternal differentiation.

The alignments included a total of 533 base pairs. The total number of polymorphic sites and their position, as well as the associated haplotypes, were determined through MEGA v. 3.1 (Kumar et al., 2004). To evaluate variance across haplotypes, a median-joining network was generated using Popart v. 1.7. (https://popart.maths.otago.ac.nz), and the clade notation was according to nomenclatures of clades reported by Liu et al. (2006). The nucleotide diversity of breeds was calculated by means of ARLEQUIN v. 3.5 (Excoffier et al., 2006). To construct the phylogenetic tree, mtDNA D-loop sequences from thirteen Swedish local chicken breeds and seventeen published reference sequences from the NCBI database (Table 1) were used. F_{ST} was computed in R using the formular $(\pi Between - \pi Within) \,/\, \pi Between$ (Hudson et al., 1992), where π_{Between} and π_{Within} is the mean number of pairwise differences between individuals from different sub-populations.

Analyzing the SNP data of eight breeds sample pools, Dalsk pärlhöna, Gotlandshöna, Öländsk dvärghöna, Hedemorahöna, Torpardvärghöna, Skånsk blommehöna, Äsbohöna, and Svarthöna, the genetic diversity was calculated based observed heterozygosity (H_o), inbreeding coefficient (F_{IS}) and the number of polymorphic information content (*PIC*). PLINK 1.90 (Purcell et al., 2007) and R (http://www.rstudio.com/) were both used to calculate H_e, H_o, and *PIC* with the default settings. Furthermore, Principal Component Analysis (PCA) was done in PLINK 1.90, and the visualization was done with R.

Table 1: Haplotype and accession numbers of chicken mtDNA D-loop sequence used in this study

Haplotype	Accession Number	Reference
A1-A2		This study
B		This study
E1-E6		This study
Liu_A1	AB114069	Liu et al. (2006)
Liu_B1	AB007744	Liu et al. (2006)
Liu_C1	AB114070	Liu et al. (2006)
Liu_D1	AY588636	Liu et al. (2006)
Liu_E1	AB114076	Liu et al. (2006)
Liu_F1	AF512288	Liu et al. (2006)
Liu_G1	AF512285	Liu et al. (2006)
Liu_H1	D82904	Liu et al. (2006)
Liu_I1	AB009434	Liu et al. (2006)
Lyimo_1	KP141690	Lyimo et al. (2015)
Lyimo_2	KP141560	Lyimo et al. (2015)
Lyimo_3	KP141460	Lyimo et al. (2015)
Lyimo_4	KP141360	Lyimo et al. (2015)
Lyimo_5	KP141260	Lyimo et al. (2015)
Lyimo_6	KP141250	Lyimo et al. (2015)
Lyimo_7	KP141240	Lyimo et al. (2015)
Lyimo_8	KP141230	Lyimo et al. (2015)

4. RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 mtDNA D-loop Polymorphic site and Population Diversity

An examination of nucleotide sequences from the mtDNA D-loop region (533 bp) revealed sixteen segregating sites (Table 2) containing transitions (A/G, and C/T). The 64 mtDNA D-loop sequences from thirteen breeds of Swedish local chickens yielded nine distinct haplotypes, with three (33.33%) being shared between populations and six (66.67%) occurring in one population each (Table 3). No haplotype was shared across all thirteen breeds. The most common haplotype (E1) was found in 39 individuals, followed by haplotypes E2 (nine individuals), E3 (seven individuals), B (three individuals), and A1 (two individuals). Additionally, four haplotypes (A2, E4, E5, E6) were represented by only a single individual.

T*able 2: Mitochondrial D-loop sequence polymorphisms identified in the different breeds of chickens*

	Nucleotide position in mt DNA															
	167	**199**	**210**	**212**	**217**	**225**	**238**	**243**	**246**	**256**	**261**	**310**	**315**	**330**	**342**	**446**
NC 007235	**T**	**T**	**C**	**A**	**T**	**C**	**G**	**T**	**T**	**T**	**C**	**C**	**T**	**C**	**A**	**C**
A1	C	.	.	G	.	T	.	.	C	.	.	.	C	.	.	.
A2	C	.	T	G	.	T	.	.	C	.	.	.	C	.	.	.
B	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
E1	.	.	.	G	C	.	.	C	C	C	T	T	C	.	.	T
E2	.	C	.	G	C	.	.	C	C	C	T	T	C	.	.	T
E3	.	.	.	G	C	.	.	C	C	C	T	T	C	T	.	T
E4	.	.	.	G	C	.	A	C	C	C	T	T	C	.	.	T
E5	.	.	.	G	C	.	.	C	C	C	T	.	C	.	.	T
E6	.	.	.	G	C	.	.	C	C	C	T	T	C	.	G	T

Table 3: mtDNA D-loop haplotypes distribution in the Swedish indigenous chickens

Haplotype	Äsbo	Bjur	Dalsk	Gamm	Gotl	Hedem	Kinda	Öländ	OlDvarg	Orus	SkBlom	Svart	Torpard	Total
A1		1					1							2
A2		1												1
B								3						3
E1	1		7	1	8	9	1		6	2	2	5		39
E2	3									2			4	9
E3							7							7
E4											1			1
E5											1			1
E6											1			1

The diversity indices for the Swedish indigenous chickens based on mtDNA D-loop is presented in Table 4. The Skånsk blommehöna had the greatest number of haplotypes and polymorphic sites at four and ten respectively, followed by Kindahöna with three haplotypes and nine polymorphic sites. Bjurholmshöns had the highest nucleotide diversity (0.0169), which was evidenced by its two samples with nine polymorphic sites and nine haplotypes (as seen in Table 4). The lowest nucleotide diversity of 0.0000 was seen in eight breeds (Dalsk pärlhöna, Gotlandshöna, Gammelsvensk, Hedemorahöna, Ölandshöna, Öländsk dvärghöna, Svarthöna, and Torpardvärghöna).

Table 4: Diversity indices of the Swedish indigenous chickens based on mtDNA D-loop

Breed	No. of Samples	No. of Haplotypes	No. of Polymorphic sites	Nucleotide Diversity
Asbo	4	2	1	0.0009
Bjurholms	2	2	9	0.0169
Dalsk	7	1	0	0.0000
Gammel	1	1	0	0.0000
Gotl	8	1	0	0.0000
Hedem	6	1	0	0.0000
Kinda	9	3	9	0.0041
Oland	3	1	0	0.0000
OlDvarg	6	1	0	0.0000
Orusthöna	4	2	1	0.0013
SkBlom	5	4	10	0.0079
Svart	5	1	0	0.0000
Torpardvarghona	4	1	0	0.0000

To investigate the differentiation among the different breeds, F_{ST} was calculated using the mtDNA d-loop data (Table 5). The values vary from 0.00 to 1. The lowest value was observed between Dalsk, Gammel, Gotl, Hedemo, ÖlDvarg and Svart, while the highest F_{ST} was between Öland and Torpard, with Dalsk, Gammel, Gotl, Hedemo, ÖlDvarg and Svart.

An analysis of molecular variance (AMOVA) (Table 6) was conducted to calculate the magnitude of the genetic diversity that is inherited through the maternal line that is present within and between populations. The division of genetic diversity within and between populations showed that 42.57% of the whole genetic variation was within individual populations, while 57.43% was observed between the different groups of chickens.

Table 5: Pairwise F_{ST} estimate between breed using mtDNA D-loop data

	Äsbo	Bjur	Dalsk	Gammel	Gotl	Hedemo	Kinda	Öland	ÖlDvarg	Orust	Skblom	Svart
Bjur	0.34											
Dalsk	0.75	0.97										
Gammel	0.75	0.97	0.00									
Gotl	0.75	0.97	0.00	0.00								
Hedemo	0.75	0.97	0.00	0.00	0.00							
Kinda	0.62	0.79	0.73	0.73	0.73	0.73						
Öland	0.55	0.83	1.00	1.00	1.00	1.00	0.85					
ÖlDvarg	0.75	0.97	0.00	0.00	0.00	0.00	0.73	1.0				
Orust	0.13	0.44	0.50	0.50	0.50	0.50	0.48	0.59	0.50			
Skblom	0.29	0.50	0.37	0.37	0.37	0.37	0.44	0.63	0.37	0.09		
Svart	0.75	0.97	0.00	0.00	0.00	0.00	0.73	1.00	1.00	0.50	0.37	
Torpard	0.25	0.83	1.00	1.00	1.00	1.00	0.85	1.00	1.00	0.50	0.625	1.00

Table 6: Analysis of molecular variance (AMOVA) of mtDNA D-loop sequences of Sweden local chickens using Arlequin

Source of variation	d.f	Sum of squares	Variance component	Percentage of Variation	Fixation Index FST
Among population	12	41.324	0.617 Va	57.43	
Within population	51	23.317	0.457 Vb	42.57	
Total	63	64.641	1.074		0.574

4.1.2 Network profiles of the clades

Figure 1. illustrates the division of clades in the chicken breeds studied, there were three distinct clades which are clade A, B, and E, formed by the nine haplotypes in this study. The naming convention for the clades utilized in this study adhered to the clade nomenclature established by Liu et al. (2006), where domestic chickens were categorized into nine distinct clades, designated as A to I based on their association with areas of domestication and the geographical dispersal. Clade E was the most common and contained eleven of the breeds, with E1 being the largest subgroup at 67.24%. Clades A and B, however, were only represented by a small number of individuals, three each. Clade A had two breeds, and clade B only contained a single breed. Of the sixty-four chickens, the largest clade was represented by 58 chickens, while the others are shared between clades A and B.

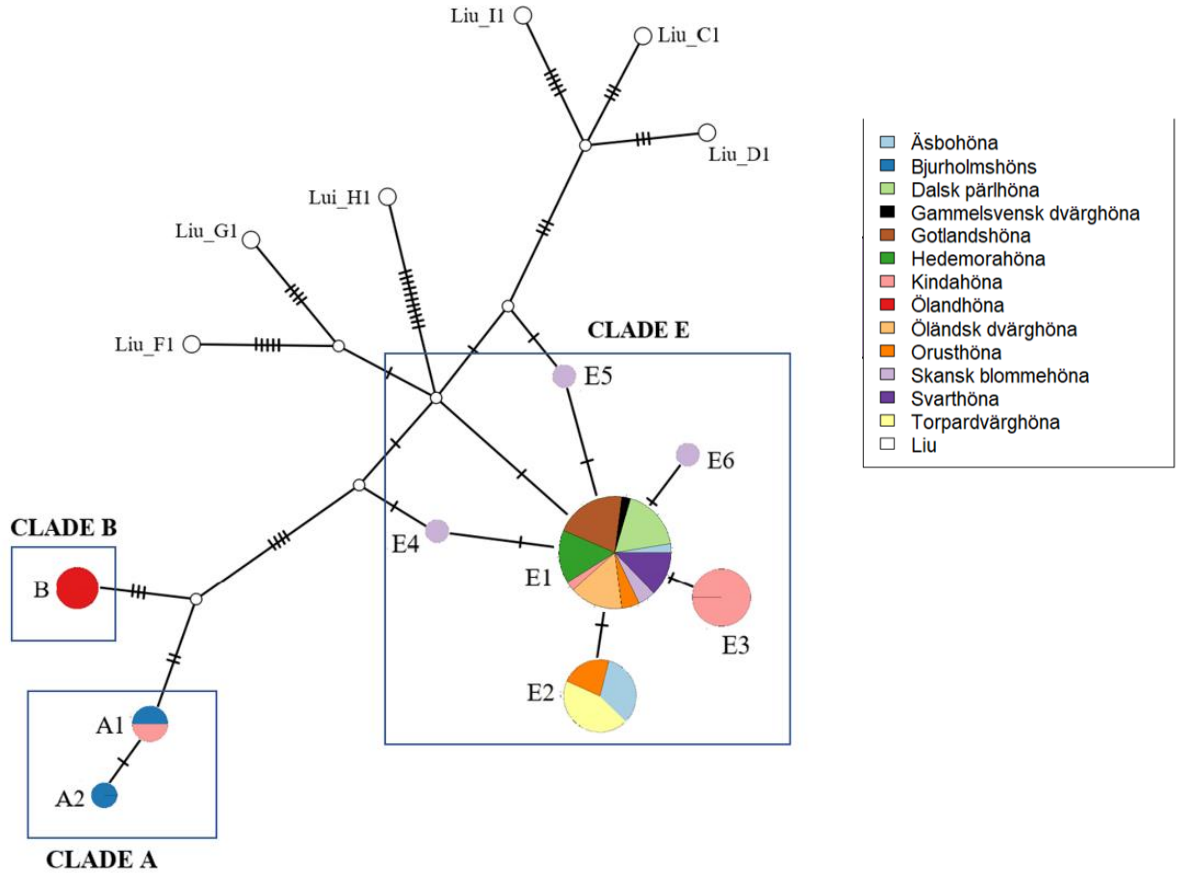

Figure 1. Median-joining haplotype network of the mtDNA D-Loop observed in this study merged with major haplotypes from Liu et al. (2006).

The neighbor-joining tree in Figure 2 showed that the mtDNA D-loop sequence data from the Dalsk pärlhöna, Gotlandshöna, Hedemorahöna, Gammelsvensk Öländsk dvärghöna, Svarthöna, Orusthöna, Torpardvärghöna, Kindahöna, one each of Skånsk blommehöna and Bjurholmshöns is clustered with the haplotype in the clade E (Liu et al., 2006) and six haplotypes from Lyimo et al. (2015). The chickens from the Ölandshöna breed were grouped with the haplotype in clade B (Liu et al., 2006), while one chicken each of Bjurholmshöns, Kindahöna, and Skånsk blommehöna breed, is clustered with the haplotype in Clade A from Liu et al. (2006) and one haplotype sample from Lyimo et al. (2015).

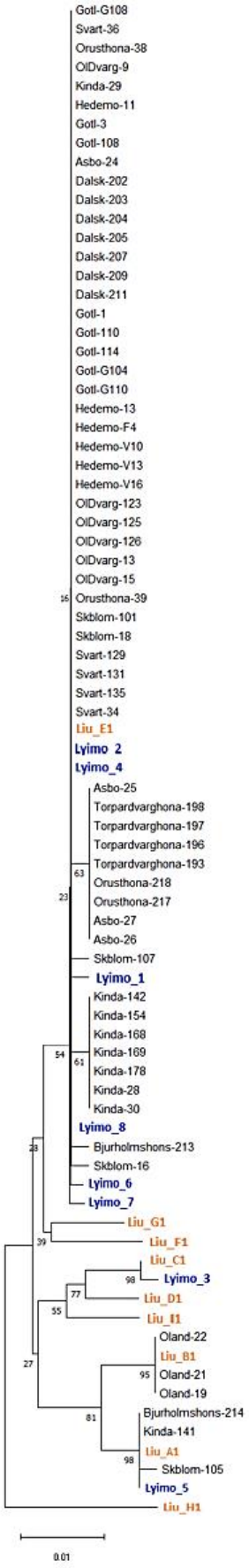

Figure 2. Neighbor-joining tree of mtDNA D-Loop sequence of Swedish local breeds of chicken. The Swedish samples are written in black, while the reference sequence from Liu et al. (2006) which represents samples from Asia are written in brown, and the reference sequence from Lyimo et al. (2015) which represents samples from Europe, are written in blue. Numbers on the tree represent local bootstrap values from 500 replicates and the scale bar represents branch length in terms of nucleotide substitution per site.

4.1.3 Genetic Diversity in Swedish local chicken using SNP Data

The results from the SNP data analysis showed that the Kindahöna chicken breed possessed the most genetic diversity (Figure 3) with the H_o of 0.62, a F_{IS} of -0.48, and a low *PIC* of 0.09 (Table 7). The *PIC* was highest in Skånsk blommehöna (0.27), and the lowest H_o was observed in Hedemorahöna chicken (0.29). The PCA (Figure 4) illustrated that five breeds (Kindahöna, Skånsk blommehöna, Ölandhöna, Öländsk dvärghöna, Äsbohöna) clustered together, while three breeds (Hedemorahöna, Svarthöna, Gotlandshöna) were separate.

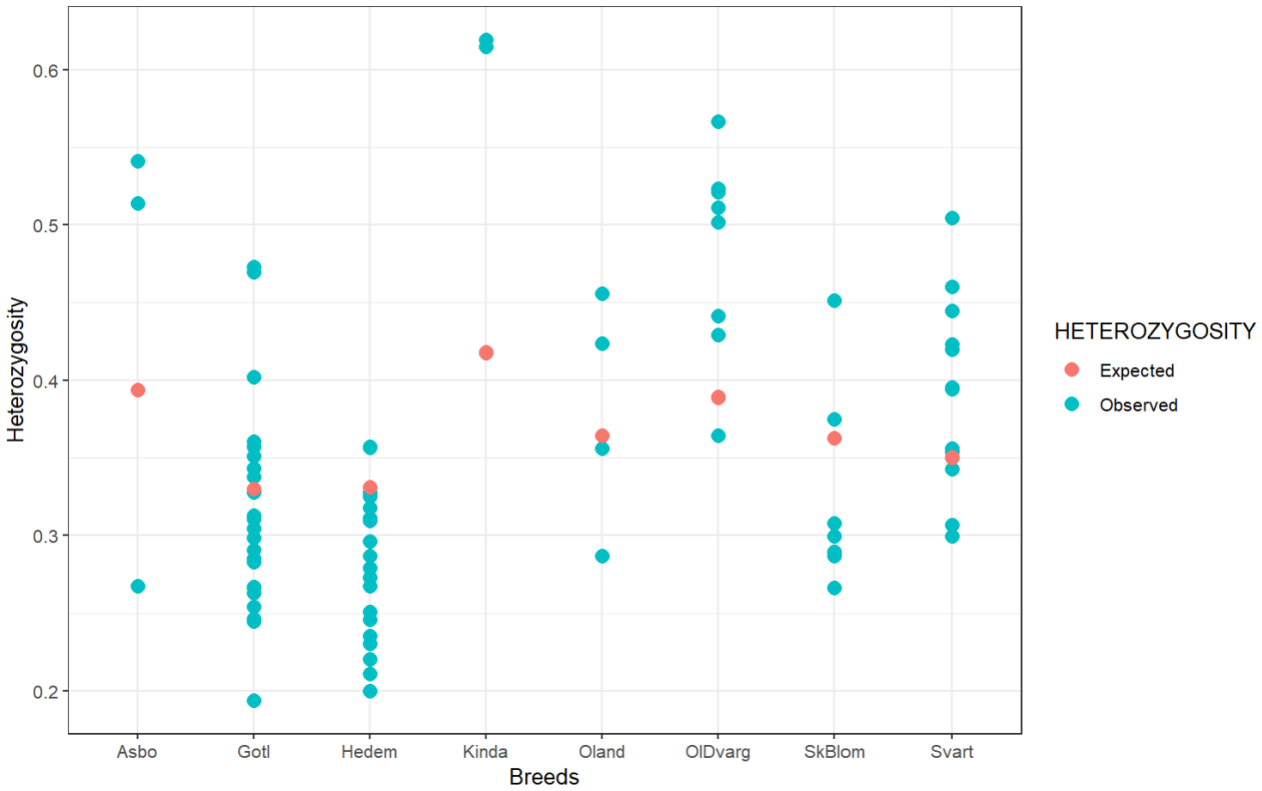

Figure 3. Dotplot of Observed and Expected Heterozygosity in the Swedish indigenous chicken breeds.

Table 7: Genetic diversity measures for the study breed

Breed	Sample size	H_o	H_e	FIS	PIC
Asbo	3	0.44	0.39	-0.12	0.17
Gotl	24	0.31	0.33	0.04	0.23
Hedem	22	0.29	0.33	0.14	0.20
Kinda	2	0.62	0.42	-0.48	0.09
Oland	4	0.38	0.36	-0.04	0.15
OlDvarg	8	0.48	0.39	-0.24	0.11
SkBlom	8	0.32	0.36	0.12	0.27
Svart	12	0.39	0.35	-0.12	0.15

O(He): Observed Heterozygosity
E(He): Expected Heterozygosity
FIS: Inbreeding coefficient
PIC: Polymorphic Information Content

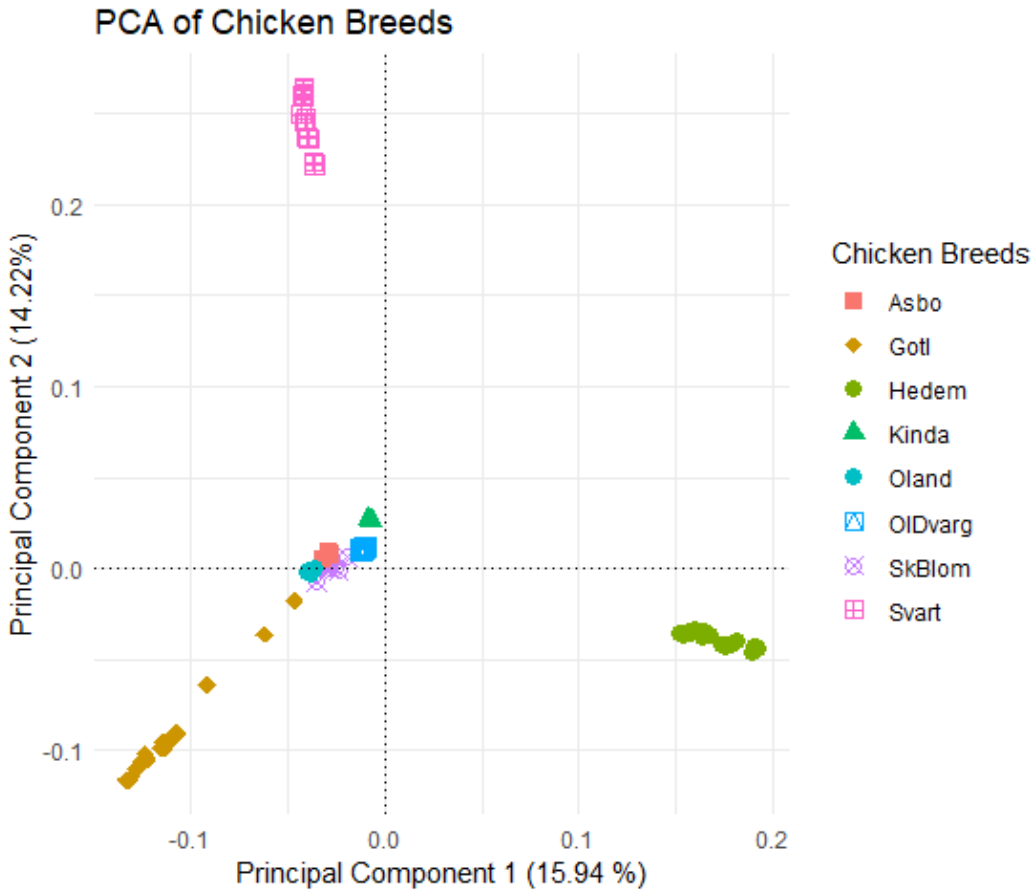

Figure 4. PCA plot of the study breed using SNP data

4.2 DISCUSSION

In this study, the hypervariable region of the partial mitochondrial DNA D-loop was studied in 64 Swedish local chickens. This region is known to contain a high amount of genetic variability, making it a suitable basis for examining population structure and evolutionary connections. The purpose of the study was to assess genetic diversity among thirteen breeds, identify the maternal genetic background, and elucidate the phylogenic relationship and clades.

The range of segregating sites in the present study lies between 167 - 446 bp, and this agrees with prior studies (Adebambo et al., 2010; Boudali et al., 2020) which have demonstrated that segregating sites found in the D-loop are typically between 133-391 bp, suggesting a higher mutation rate in this area. However, Gao et al. (2017) revealed more polymorphic sites beyond 446 bp after examining the full sequence of the D-loop, indicating that the full mtDNA D-loop sequence may be a helpful source of genetic markers for studying population genetics and tracing the ancestry of animals.

The average nucleotide diversity of Swedish local chicken breeds was 0.0024, which is higher than the values reported in Albania (0.0004), Turkey (0.0007), Nigeria (0.0016), and Sudan (0.0018), but lower than those in Kenya (0.0119), Algeria (0.003), and Ethiopia (0.0032). Nevertheless, it is comparable to the figure of 0.002 in Iran (Adebambo et al., 2010; Hassaballah et al., 2015; Mwacharo et al., 2011). The Bjurholmshöns breed showed the highest nucleotide diversity (0.0169) (Table 4) with two individuals, nine polymorphic sites and two haplotypes, whereas the Skånsk blommehöna breed had the second highest nucleotide diversity of 0.0079, with five individuals, ten polymorphic sites and four haplotypes. The findings of this study suggests that the Bjurholmshöns breed and the Skånsk blommehöna have a higher level of polymorphism than the other Swedish local chicken breeds. Eight of the breeds showed the lowest level of nucleotide diversity and had only one haplotype each, which is similar to what Liu et al. (2004) discovered with some Chinese breeds. This could be associated with the geographic distance from the original domestication region, as most of the breeds (Dalsk pärlhöna, Gammelsvensk dvärghöna, Hedemorahöna, Svarthöna and Torpardvaghöna) with low diversity are located not far from each other in the mainland of Sweden, but with some exceptions (Ölandshöna, Öländsk dvärghöna and Gotlandshöna), which are Island breeds and may have contributed to their genetic isolation and reduced genetic diversity, combined with the cultural practice of raising chickens in small, isolated populations (Granevitze et al., 2007; Groeneveld et al., 2010), or due to selective breeding which might have caused a decrease in diversity, however, we were unable to find any literature information on selective breeding for either of the breeds.

In accordance with Lyimo et al. (2015), who found that chicken breeds in Europe were separated into five clades, (A to E), the Swedish local chicken populations were also seen to assign into three of these clades (A, B, and E). Boudali et al. (2020) also discovered that the Algerian indigenous local chickens have two haplogroups, A and E. Most Swedish local chickens had mtDNA haplotypes that belonged to clade E, similar to the observations made by Lyimo et al. (2015) on European chicken breeds, Meydan et al. (2016) on Turkish and Iranian native chickens, and Boudali et al. (2020) on Algerian domestic chickens. In contrast, Gao et al. (2017) noticed that clades A and B were the dominant types in the Jiangxi chicken breeds from China. This Clade E is said to be the most widespread clade of domestic chickens, and is seen in India, the Yunnan province of China, Japan, Indonesia, the Middle East, and Europe (Liu et al., 2006; Gongora et al., 2008). The clade has also been identified in Hungarian, Nigerian, and Vietnamese local chicken breeds (Revay et al., 2010; Adebambo et al., 2010; Cuc et al., 2010). Lyimo et al., (2015) and Liu et al., (2006) suggests that the Indian subcontinent is the original source of the haplogroup E found in Europe. Our study results indicate a dominant presence of this clade in Swedish local chicken breeds, suggesting an Indian subcontinent origin for most of the breeds. It is widely believed that most European chicken breeds originated from the subcontinent of Indian, where haplogroup E is prevalent.

Clades A and B were identified in the study. Clade B is specific to the Olandhöna breed and Clade A consists of two breeds, Bjurholmshöns and Kindahöna, with Bjurholmshöns being unique to this clade. Clade A has been earlier found by Liu et al. (2006) in Yunnan province of China and Japan, Madagascar (Razafindraibe et al., 2008), in Zimbabwe (Muchadeyi et al., 2007), and in Vietnam by Cuc et al. (2010). Gao et al. (2017) reported that clades A and B are present worldwide, except in Africa and certain regions of Asia such as Bangladesh (Islam et al., 2019). This study found 4.69% each of clades A and B, suggesting that these clades also slightly influenced Swedish local chickens. Based on the research conducted by Liu et al. (2006) and supported by the findings of Gongora et al. (2008) it is probable that these clades originated in the region of Yunnan in China and its surrounding areas. The presence of these clades in the study could be due to the introduction of chickens into Sweden through various migration routes or international trade. The occurrence of these clades in the study may be a result of chickens being introduced to Sweden through diverse migration paths or global commerce. The existence of clades A, B, and E in Swedish chickens corresponds to historical documentation of chicken migration from the eastern and southern Asian areas by two significant trading paths (West and Zhou 1988; Crawford 1995; Tixier-Boichard et al., 2011).

An intriguing finding is that the sequences of the mtDNA D-loop showed that the Ölandshöna and Bjurholmshöns breeds were distinct from other Swedish breeds (Figure 1). This may be due to the origin of the Ölandshöna breed, which is from

an island in the Baltic Sea, indicating that its maternal lineage may not have originated from Sweden mainland, but may have been brought to the island by the Vikings through transportation by ship. The Bjurholmshöns breed is thought to have come from the Bjurholm municipality in Northern Sweden, which is situated far away from the other parts of Sweden. This distance may have resulted in the development of specific genetic traits not found in other breeds, apart from the Kindahöna which has a similar haplotype to the Bjurholmshöns.

The F_{ST} analysis indicates that certain breeds have a close genetic relationship to one another, suggesting that the breeds may have the same maternal origin, while some extreme genetic difference from each other which may be as a result of the distant location of these breeds, notably, the Bjurholmshöns and Ölandshöna breeds, geographically isolated and situated at a considerable distance from other breeds, display significantly lower levels of genetic relatedness with nearly all other breeds. This supports the claim made by Nie et al. (2019) that if populations are separated by a significant distance, they may have less genetic exchange and over time, genetic differences can accumulate, leading to a higher genetic variation between the populations. However, this finding is limited by small sample size per breed, and with a small sample size, the estimate of F_{ST} may be influenced by rare allele, which can lead to an overestimation or underestimation of differentiation.

The phylogenetic relationship analysis using mtDNA D-loop polymorphism (Figure 2) reveals a distinct grouping of the local breeds of chicken in Sweden with several of the European samples (Lyimo et al., 2016) included in the study (Table 1), but only three of the nine Asian samples (Liu et al., 2006). This is likely due to the fact that chickens have been domesticated and traded across Europe for thousands of years, leading to potential genetic exchange between European regions and populations, and resulting in a clear genetic differentiation from Asian breeds.

The results of the analysis of molecular variance indicate that the major source of genetic diversity in Swedish local chicken breeds is the variation among populations (57.43%). Although there was a significant amount of genetic variation within different chicken breeds, the variation between breeds was greater than within breeds, suggesting greater genetic exchange within a breed than between breeds. This highlights the importance of preserving genetic diversity both within and between populations for effective management and conservation strategies (Ceccobelli et al., 2015).

The analysis of the autosomal SNP data revealed that chickens of all breeds have sustained a considerable amount of genetic diversity in terms of heterozygosity (H_o and H_e). The chicken breeds, employed in the study, had a greater genetic diversity (H_o) than the North American and Chinese breeds (Lujiang et al., 2006; Zhang et al., 2018) which may imply that the Swedish local chicken breeds have been

subjected to less intense selection. The Kindahöna breed showed potential for high genetic diversity with the highest (H_o) and lowest inbreeding coefficient. However, the conclusions on its genetic diversity are limited by the low sample size of only two samples. The Hedemorahöna breed had lower genetic diversity, in this study with the lowest H_o and highest inbreeding coefficient, the breed had a considerable sample size, and we could say it probably reflects the genetic variability of the breed chicken but may not be the breed with the lowest diversity among the Swedish breeds because of the influence of low sample size in the other breeds. The range of estimated inbreeding coefficients (-0.48 to 0.14) in the study is within the acceptable range for maintaining genetic diversity, which aims to preserve 90% of the initial population's genetic diversity and an inbreeding coefficient below 0.1 over 100 years (Wu, 2016). A negative inbreeding coefficient indicates that the individual or population has less genetic homozygosity than would be expected if the population was randomly mating. The study found that the rate of genetic variation among the Swedish local chicken breeds varied.

The principal component analysis using the autosomal SNPs indicated that the Ölandshöna, Skånsk blommehöna, Öländsk dvärghöna, Äsbohöna, and Kindahöna breeds are more genetically similar to each other than with other breeds, as they cluster together in the PCA plot. On the other hand, Gotlandshöna, Hedemorahöna, and Svarthöna showed clear genetic differentiation from the other Swedish local chicken breeds. This finding differs from the mtDNA D-loop analysis, where the Ölandshöna breed appeared distinct from the other breeds. The observed inconsistency may be explained by the fact that the autosomal SNP information reflects the entire genetic diversity of the population, which encompasses both maternal and paternal lineages. This implies that the introduction of male genetic material from other regions of Sweden might have influenced the overall genetic variation.

A limitation of the study is that the sample size per breed is low, potentially leading to an oversimplified or distorted understanding of the population's evolutionary history. Other haplotypes may exist in some breeds that were not detected due to the small sample size, however, despite this, important conclusions can still be drawn from the study.

5. CONCLUSION

The mitochondrial DNA analysis conducted in this study indicates that the local chicken breeds in Sweden have several maternal origins, likely originating from the Indian subcontinent, Yunnan, and surrounding regions of China. The presence of identical haplotypes in nearly all breeds suggests a common ancestry or genetic exchange among them, and some breeds exhibit a genetic relationship that may indicate interbreeding. The study also found a significant level of genetic variation among certain breeds, while others have low levels. To further understand the genetic diversity of these breeds, a larger sample size for each breed should be used in future studies.

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Popular science summary

The genetic information carried by individuals within a population and between populations is known as genetic diversity. Genetic diversity is important for enabling individual populations to adapt and survive in changing environments, and it may contain genetic variants that improve resistance to diseases. In this study, a genetic analysis was conducted to evaluate genetic diversity, origin, and relationships among Swedish local chicken populations. Two sets of data were used in this research: one consisted of the genetic material which is inherited only maternally (mtDNA D-loop), for 64 individuals from 13 different breeds of Swedish local chickens, while the other included genetic material which is inherited from both parents (single nucleotide polymorphisms (SNPs)) for 83 individuals from eight of the breeds. The results of the analysis of the maternal genetic data showed that there was more genetic variation between different chicken breeds than within the same breeds. However, in a previous study, domesticated chickens were classified into nine separate groups (A to I) based on their region of origin and spread across the globe, and most of the breeds in this study were found to belong to group E, while others belong to groups A and B. This suggests that the Swedish local chickens have several maternal lineages that likely originated from the Indian subcontinent, Yunnan province, and the regions of China. Using the maternal genetic data, it was also discovered that Swedish local chicken breeds were more closely related to European chicken breeds than to Asian breeds. Analysis of the genetic material from both parents revealed a closer genetic relationship among five of the Swedish local chicken breeds, while the other three breeds were observed to be distinct. The results of this study can help improve how we breed and preserve Swedish local chicken breeds. Analysing both types of data used in this study is recommended, to gain a more comprehensive understanding of genetic diversity, origin, and relationships of animals.

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