

Polymorphisms Of the QTL Region Associated with Shank Feathering in Chicken

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Abstract

A total of twenty-six local chickens were representing shank feathering and non-feathering shank were used to sequence five QTLs, which associated with shank feather trait in chicken. The five location sequence results were shown polymorphism between the shank feathering and non-feathering shank. All the candidate markers were differed between the shank feather and non-feathering shank. The big distance was in (ADL221), and the less distance was in marker MCW315.

Keywords: Shank feather, microsatellite, QTL, DNA, and Sequencing.

Introduction

Shank feathering or “ptilopody” is a dominant trait (1) caused by two genes (2), named Pti-1 and Pti-2 (3). This trait was found in several bird species like chicken (4), pigeon (5), and raptor (6). Several studies investigated the relationship between the egg traits and the appearance of shank feather in chicken, (7) found that there was a difference between shank feathering chicken and non-feathering shank chicken in their external egg traits. Other studies by (8-9) displayed a significant difference in internal egg traits. Furthermore, (10) revealed that egg traits remain without differences between pre- and post-molting. Moreover, this trait was used to classify chicken in several places such as Algeria (11), and India (12).

With the development of molecular genetics, it is possible to know the genetic location of the studied traits, and can also study the sequence of amino acids of the gene (13). In (14) study found two QTL region candidates for shank feathering genes located in chromosomes 13 and 15, in the positions 55/1.56 and 47/12.22 respectively. The objective of this study is to detect the polymorphism of the candidate QTLs for the shank-feathering gene in two Kurdish local chicken breeds.

Materials and Methods

The present work was done in September 2018 in the animal science department laboratories, college of agricultural engineering sciences in University of

Sulaimani. Blood samples were collected from animal production department in the directorate of agricultural research in Sulaimani, which associated with ministry of Agriculture in KGR-Iraq. A total of 26 local chickens were representing shank feathering (SF=13) and non-feathering shank (NFS=13). The chickens used were described by (7). 2.5 ml of fresh blood samples were taken from wing vein from each individual in and collected in tubes contains EDTA anticoagulant. The blood was gently mixed, and kept on box with ice bag until transporting to the laboratory and stored it in the refrigerator at -20°C until the isolation of genomic DNA.

Genomic DNA was isolated using a commercial kit, AccuPrep® Genomic DNA Extraction kit with slight modifications. Then the DNA samples qualification and concentration were evaluated by spectrophotometer (Nano-Drop2000, Delaware USA), based on 260 and 280 nm absorbance, and agarose gel electrophoresis analysis.

Five polymorphic microsatellite markers, which were located on the both chromosome 13, and 15 where mapped (Table 1) to studied the polymorphism of each marker. The PCR program were used included an initial denaturation step at 94°C for 5 min followed by 30 cycles of 94°C for 30 sec. 55°C for 30 sec., extension at 72°C for 30 sec. and final extension at 72°C for 10 min. The PCR result were separated by electrophoresis at (85 V) Through a 1.5% agarose-TBE gel depending on the fragment

sizes for 90 Min. Ethidium Bromide staining was used for visualization under UV light. For determining the nucleotide sequence of DNA Sanger, sequencing method was applied. Phylogenetic tree was performed using MEGA 6.06.

The phylogenetic tree shown in figure.1 determines the genetic distances between the shank feather and non-feathering shank based on the Microsatellite marker (MCW 322). The phylogenetic tree grouped into 2 main clusters, the first cluster divided to two sub-clusters; the first one included the non-feathering shank female (NFS Female) and the shank feathering female (SF Female). The second sub-cluster included just the shank-feathering male (SF Male), as for the second cluster included the non-feathering shank male (NFS male).

The phylogenetic tree shown in figure.2 determines the genetic distances between the shank feather and non-feathering shank based on the Microsatellite marker (ADL 225). The phylogenetic tree grouped into 2 main clusters, the first sub-cluster included the non-feathering shank male (NFS Male) and the shank feathering male (SF Male). The second sub-cluster included the non-feathering shank female (NFS female) and the shank feathering female (SF female).

The phylogenetic tree shown in figure.3 determines the genetic distances between the shank feather and non-feathering shank based on the Microsatellite marker (MCW

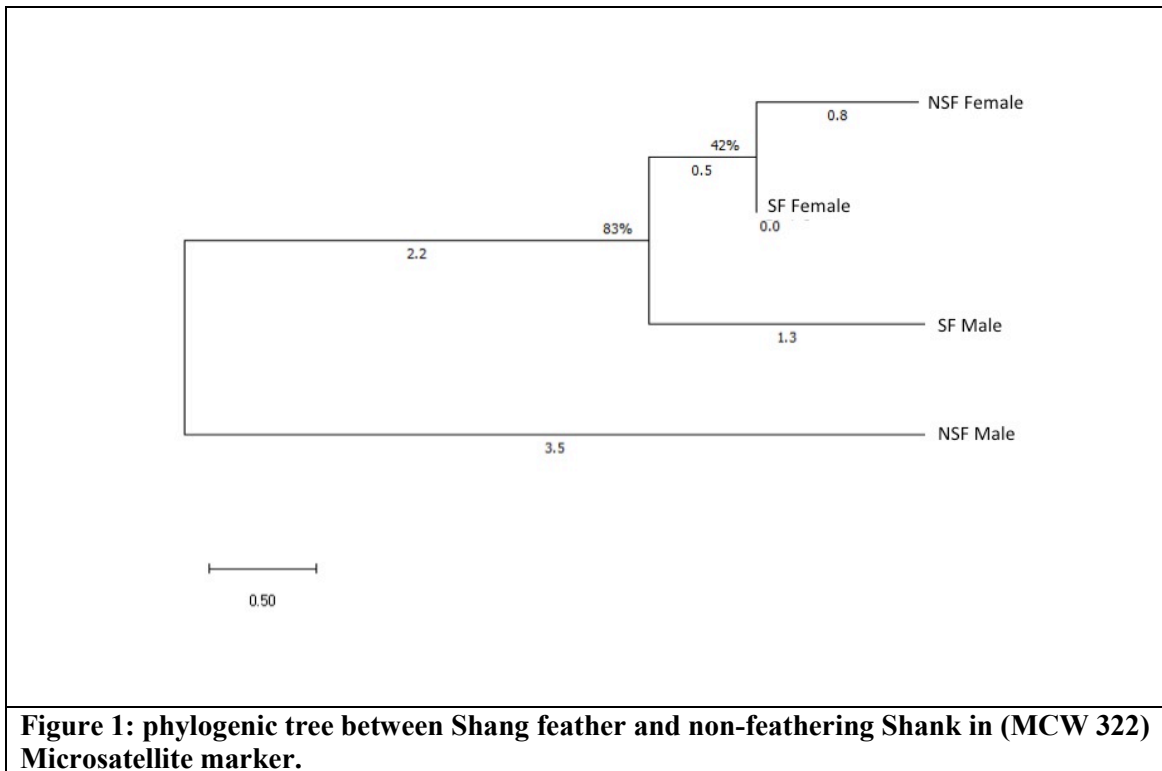
315). The phylogenetic tree grouped into 2 main clusters; the first cluster was divided into two sub-clusters. The first sub-cluster included non-feathering shank female (NFS Female), and the shank feathering female (SF Female). The second sub-cluster included just the non-feathering shank male (NFS Male). As for the second cluster just included the shank-feathering male (SF Male).

The phylogenetic tree shown in figure. 4 determines the genetic distances between the shank feather and non-feathering shank based on the Microsatellite marker (ADL 221). The phylogenetic tree grouped into 2 main clusters, the first sub-cluster included the non-feathering shank male (NFS Male) and the shank feathering male (SF Male). The second sub-cluster included the non-feathering shank female (NFS female) and the shank feathering female (SF female).

The phylogenetic tree shown in figure. 5 determines the genetic distances between the shank feather and non-feathering shank based on the Microsatellite marker (PCR WAG-110C15). The phylogenetic tree grouped into 2 main clusters, the first sub-cluster included the shank feathering female (SF female) and the non-feathering shank female (NFS Female). The second sub-cluster included the shank feathering male (SF male) and the non-feathering shank male (NFS Male).

Table 1: List of the markers used and their information

Marker name	Chr.	Position (cM)	Primer's sequence
MCW 322	13	67	F- GATCTCCCTAGCTACAAACC R- CTTCCGCCTTCTTGAGAGTC
ADL 225	13	70	F- CCAAAAAGCTGTATCACCTT R- GCCTGTTGTAAACCACCTGA
MCW 315	13	60	F- TGATGCTGGAGGCAAACATC R- GATCCAAGCCTGGAAGTATG
ADL 221	15		F- GTTCCAATGCCCCCTAATGC R- GTGTGCCCGTAATCCTGTAT
PCR WAG-110C15	15		F- ATTTCTCCAACGTTCCCAAG R- GTGGGCTCCTCTTCTCTTTG


Figure 1: phylogenic tree between Shang feather and non-feathering Shank in (MCW 322) Microsatellite marker.

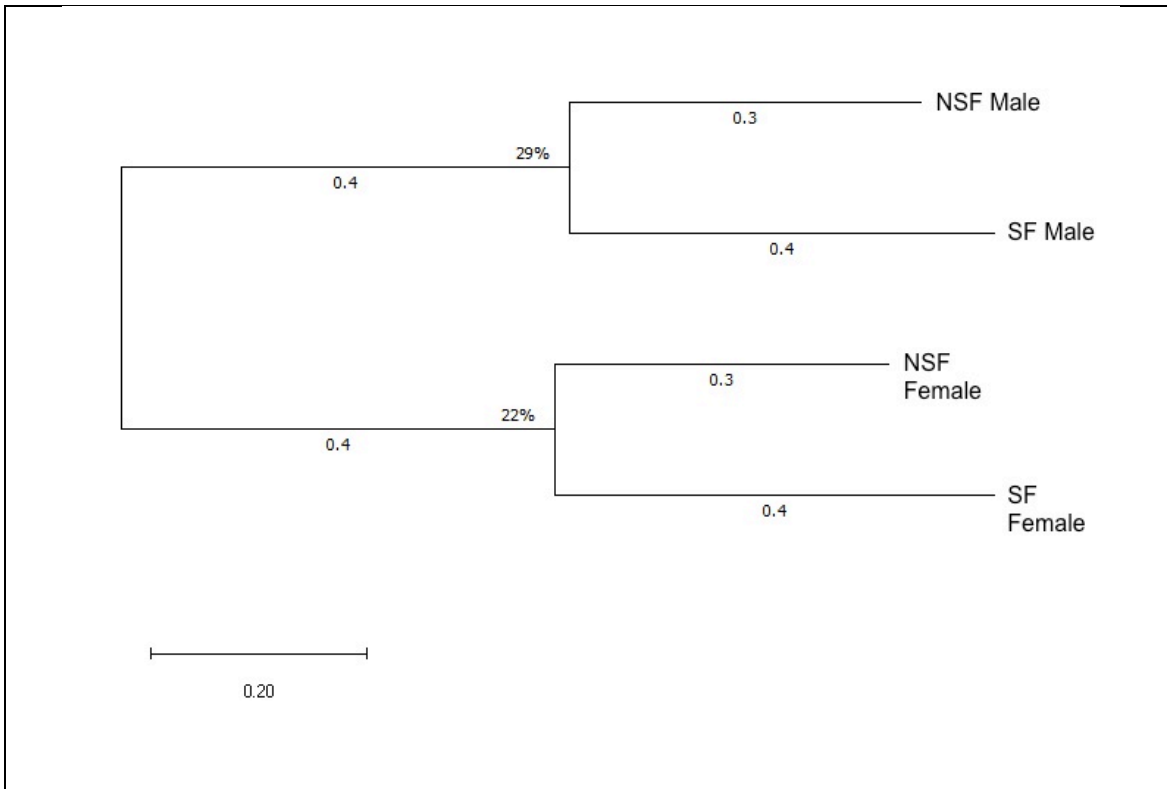


Figure 2: phylogenic tree between Shang feather and non-feathering Shank in (ADL 225) Microsatellite marker.

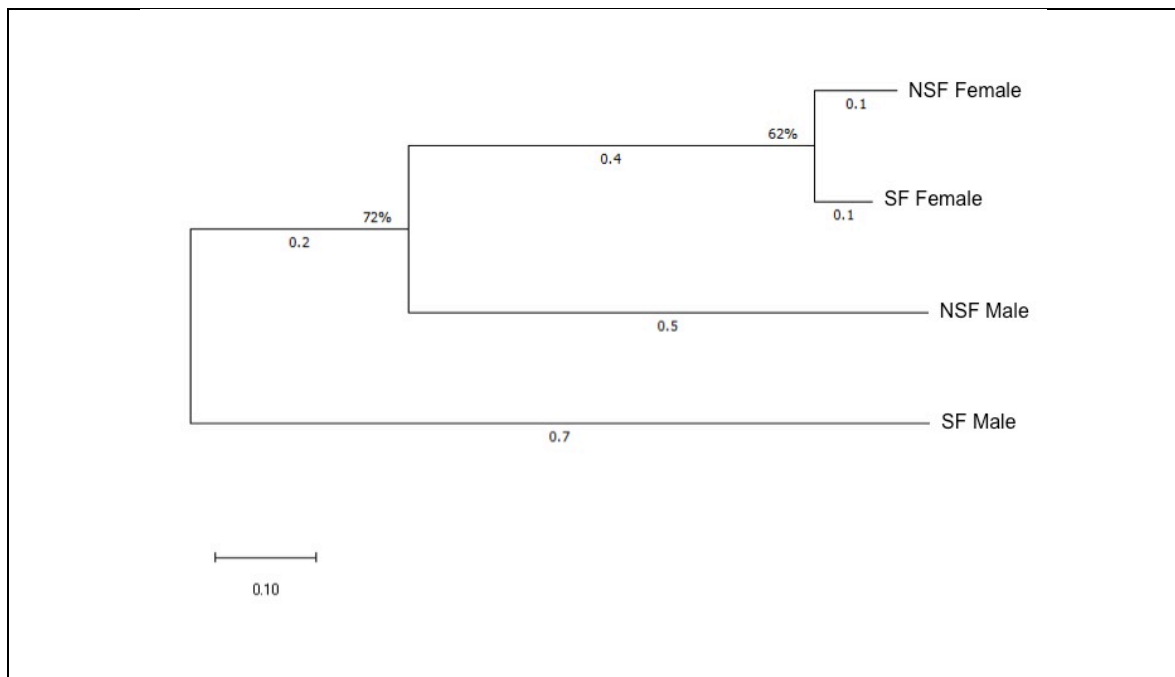
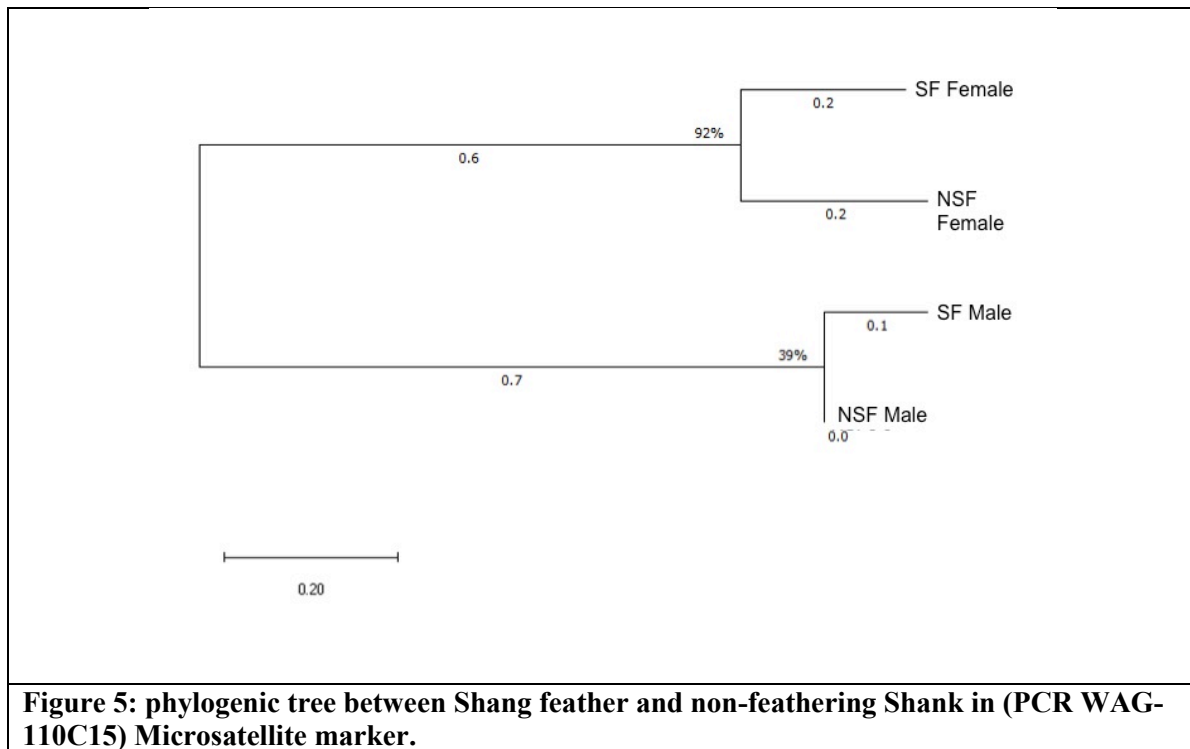
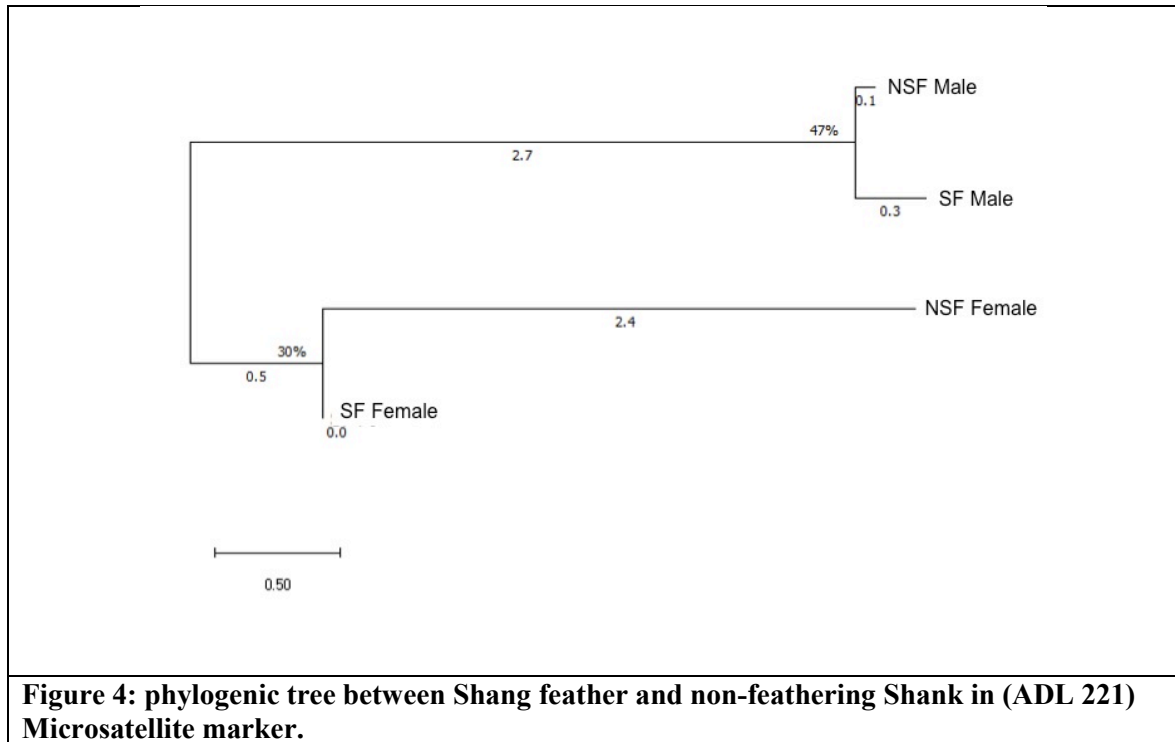


Figure 3: phylogenetic tree between Shang feather and non-feathering Shank in (MCW 315) Microsatellite marker.



Discussion:

In the Dendrogram tree the male and female for the both shank feather and non-feathering shank lines are not related to chicken gender. Also, the present study showed no close relationship to each other for the five microsatellites. The big distance was found in (ADL221) marker, which it could be the candidate marker to the studded trait. Several studies were done to analysis the relationships among the breeds, (15) was used forty microsatellite markers to study the genetic relationship among the Japanese long-tailed chicken breeds. In the study of (16) also used 25 microsatellite markers to study the genetic diversity among Saudi native chicken populations and reported its useful tools to study the conservation of diversity native chicken. Moreover (17) used exploited microsatellite markers to classify the chicken into groups according to their genetic similarity, and regarded as suitable tools to group the chickens according to the similarity in order to support the selection.

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