# Retrotransposons Mobility in Eight Pure Lines of Laying Chickens

**Cihat Erdem Bulbul** ( cihaterdem.bulbul@omu.edu.tr )
Ondokuz Mayis University
**Fatih Bilgi**
Ondokuz Mayis University
**Levent Mercan**
Ondokuz Mayis University
**Sevgi Marakli**
Yildiz Technical University

**Research Article**

**Keywords:** Chicken, pure line, Copia-like, Nikita, SIRE, Sukkula

**Posted Date:** March 30th, 2023

**DOI:** https://doi.org/10.21203/rs.3.rs-2747096/v1

# Abstract

In this study, retrotransposons mobility in eight pure lines of the domestic chicken (*Gallus gallus domesticus*) was analysed using IRAP-PCR (Inter-retrotransposon amplification polymorphism Polymerase Chain Reaction) method. Polymorphism rates were detected as 0–78% in *Copia-like*, 0–73% in *Nikita*, 0–60% in *SIRE*, and 0–38 in *Sukkula* in Rhode Island Red, Barred Rock, Colombian Rock, Line-54, Black Line, Blue Line, Brown Line, and Maroon Line chickens. *In silico* analyses presented that Blue Line with Line-54 and Barred Rock with Colombian Rock are closely related regarding these four retrotransposons. These horizontally transferred plant-specific retrotransposons may affect changes in the pure-line chicken genome.


# Introduction

Recent assumptions about chicken domestication are based on rice (*Oryza* sp.) cultivation in central Thailand around 3500 years ago because cultivated rice attracted wild ancestors of the domestic chicken. Also, several studies have reported a correlation between the spread of domestic chicken and cereal farming in Asia, Africa and Europe (Peters et al., 2022).

Transposable elements (TEs) may jump across the genome by duplicating and splicing themselves. TEs are classified according to the transposition mechanisms. Class I elements require an RNA intermediate to duplicate themselves within a genome. In contrast, Class II elements replicate themselves directly without an intermediary via cut-and-paste mechanism (Kim et al., 2022). The predominant class of TEs can vary significantly among taxa and species in terms of frequency, location, and activity levels (Serrato-Capuchina & Matute, 2018). Species are separated from one another by pre- and postzygotic barriers. Prezygotic barriers are sperm/egg incompatibility, and postzygotic barriers involve various fitness reductions. TEs have been found to affect a trait potentially involved in reproductive isolation in interspecies crossbreds. Besides, TEs affect yield traits, including reproduction in intraspecies crossbreds (Serrato-Capuchina & Matute, 2018).

Rhode Island Red (RIR) is a chicken breed used for egg-laying (i.e. egg-type) purposes. Although it was used for meat (i.e. meat-type) purposes in old generations, nowadays, it is mainly raised for egg production. Barred (Plymouth) Rock (BAR) and Colombian (Plymouth) Rock (COL) are chicken breeds that are raised for both meat and eggs (i.e. combine-type). BAR is also suitable for cold environmental conditions. RIR, BAR, COL, and Line-54 (L-54) breeds are brown egg-laying chickens. Black Line (BLA), Blue Line (BLU), Brown Line (BRO), and Maroon Line (MAR) breeds are white egg-laying chickens (Göger et al., 2017). L-54 and BLA, BLU, BRO and MAR are pure lines bred at the Poultry Research Station within the scope of the “Poultry Research and Development Project”. This study was conducted to determine the polymorphisms of plant-specific *Copia-like*, *Nikita*, *SIRE*, and *Sukkula* retrotransposons in eight different pure chicken lines including RIR, BAR, COL, L-54, BLA, BRO, MAR ve BLU by using IRAP marker technique. These retrotransposable elements could assumed important roles in shaping the structure and function of the chicken genome over time.

## Material And Methods

# Obtaining samples and genomic DNA isolation

The blood samples of chicken lines RIR, BAR, COL, L-54, BLA, BRO, MAR and BLU were collected from Poultry Research Station, Ankara-Turkey. The samples that were granted permission by the OMU Local Ethics Committee for Animal Experiments with decision number 1 on 6.11.2012 were used for this study. Genomic DNAs of four chickens belonging to each line were isolated using the salting-out method (Miller et al., 1988). The qualitative and quantitative measurements of gDNAs' were analysed using agarose gel and a spectrophotometer (NanoDrop®, Thermo, USA), respectively.

Copia-like, Nikita, SIRE, **and** Sukkula **IRAP-PCR analyses**

IRAP-PCR analyses were performed according to Kalendar & Schulman (2006). PCR assays were performed in the T100 Thermal Cycler (BIO-RAD, USA). Amplification of the reactions was optimised in a final volume of 20 µL containing 4 µL ultrapure sterile water, 10 µL PCR master mix (ABT 2X PCR MasterMix), 2 µL of primer (1 µM/µL), and 4 µL of template genomic DNA (4 µM/µL). Final concentrations were indicated in parenthesis. Primer sequences are 5GGGGCTTGGTTCGAAAGGTTT3' for *Copia-like* F and 5'TCTGAGGCAAGACGTTCCTT3' for *Copia-like* R, 5'ACCCCTCTAGGCGACATCC3' for *Nikita* (Leigh et al., 2003), 5'CAGTTATGCAAGTGGGATCAGCA3' for *SIRE* (Chesnay et al., 2007) and 3'GGAACGTCGGCATCGGGCTG5' for *Sukkula* (Leigh et al., 2003). PCR conditions were as follows: one initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 40°C for *Copia-like*, 47°C for *SIRE*, 50°C for *Sukkula* and 52°C for *Nikita* for 30 sec and extension at 72°C for 1 min. The final elongation step was performed at 72°C for 10 min. The amplification products and molecular weight marker (GeneRuler 100 bp Plus DNA Ladder, Thermo Scientific™) were resolved on 1.5% agarose gel in 1X TAE (Tris–Acetic Acid–EDTA) at 80 V for 70 min and photographed on a Gel Documentation System (BIO-RAD, USA). IRAP-PCR band profiles were evaluated visually, and polymorphisms were calculated according to Jaccard's coefficient (1908) in all samples (Jaccard, 1908).

# Construction of phylogenetic tree

The phylogenetic tree was constructed by evaluating the retrotransposon band profiles in agarose gels via GelJ v.2.0 software. The dendrogram was constructed by UPGMA (unweighted pair group method with arithmetic mean) (Heras et al., 2015).

## Results

IRAP-PCR results for *Copia-like* retrotransposon were presented in Fig. 1 and Fig. 2. Polymorphism rates were 0–60% for COL line, 0–50% for RIR line and 20–40% for BRO line. No polymorphism was detected for BAR lines (Fig. 1 and Table 1).

Table 1
Polymorphism rates for the first group in terms of *Copia-like* retrotransposon. 1–4, BAR line samples; 5–8, COL line samples; 9–12, RIR line samples; 13–16, BRO line samples

| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | - | | | | | | | | | | | | | | | |
| 2 | **0** | - | | | | | | | | | | | | | | |
| 3 | **0** | **0** | - | | | | | | | | | | | | | |
| 4 | **0** | **0** | **0** | - | | | | | | | | | | | | |
| 5 | 60 | 60 | 60 | 60 | - | | | | | | | | | | | |
| 6 | 33 | 33 | 33 | 33 | **60** | - | | | | | | | | | | |
| 7 | 50 | 50 | 50 | 50 | **50** | **20** | - | | | | | | | | | |
| 8 | 33 | 33 | 33 | 33 | **60** | **0** | **20** | - | | | | | | | | |
| 9 | 20 | 20 | 20 | 20 | 50 | 50 | 67 | 50 | - | | | | | | | |
| 10 | 33 | 33 | 33 | 33 | 60 | 0 | 20 | 0 | **50** | - | | | | | | |
| 11 | 20 | 20 | 20 | 20 | 50 | 50 | 67 | 50 | **0** | **50** | - | | | | | |
| 12 | 17 | 17 | 17 | 17 | 67 | 17 | 33 | 17 | **33** | **17** | **33** | - | | | | |
| 13 | 0 | 0 | 0 | 0 | 60 | 33 | 50 | 33 | 20 | 33 | 20 | 17 | - | | | |
| 14 | 40 | 40 | 40 | 40 | 33 | 40 | 60 | 40 | 25 | 40 | 25 | 50 | **40** | - | | |
| 15 | 33 | 33 | 33 | 33 | 60 | 0 | 20 | 0 | 50 | 0 | 50 | 17 | **33** | **40** | - | |
| 16 | 20 | 20 | 20 | 20 | 50 | 20 | 40 | 20 | 40 | 20 | 40 | 33 | **20** | **25** | **20** | - |

*The numbers in bold represent the intrapopulation polymorphism rates of the pure lines.

We detected 0–78% polymorphism for BLA line, 0–33% for BLU and L-54 lines. There is no *Copia-like* retrotransposon polymorphism in MAR lines (Fig. 2 and Table 2).

Table 2
Polymorphism rates for the second group in terms of *Copia-like* retrotransposon. 1–4, BLA line samples; 5–8, BLU line samples; 9–12, L-54 line samples; 13–16, MAR line samples

| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | - | | | | | | | | | | | | | | | |
| 2 | **75** | - | | | | | | | | | | | | | | |
| 3 | **13** | **78** | - | | | | | | | | | | | | | |
| 4 | **0** | **75** | **13** | - | | | | | | | | | | | | |
| 5 | 0 | 75 | 13 | 0 | - | | | | | | | | | | | |
| 6 | 25 | 75 | 13 | 25 | **25** | - | | | | | | | | | | |
| 7 | 25 | 75 | 13 | 25 | **25** | **0** | - | | | | | | | | | |
| 8 | 33 | 63 | 22 | 33 | **33** | **13** | **13** | - | | | | | | | | |
| 9 | 38 | 71 | 25 | 38 | 38 | 14 | 14 | 25 | - | | | | | | | |
| 10 | 38 | 71 | 25 | 38 | 38 | 14 | 14 | 25 | **0** | - | | | | | | |
| 11 | 43 | 60 | 50 | 43 | 43 | 43 | 43 | 50 | **33** | **33** | - | | | | | |
| 12 | 38 | 71 | 25 | 38 | 38 | 14 | 14 | 25 | **0** | **0** | **33** | - | | | | |
| 13 | 44 | 57 | 33 | 44 | 44 | 25 | 25 | 13 | 14 | 14 | 43 | 14 | - | | | |
| 14 | 44 | 57 | 33 | 44 | 44 | 25 | 25 | 13 | 14 | 14 | 43 | 14 | **0** | - | | |
| 15 | 44 | 57 | 33 | 44 | 44 | 25 | 25 | 13 | 14 | 14 | 43 | 14 | **0** | **0** | - | |
| 16 | 44 | 57 | 33 | 44 | 44 | 25 | 25 | 13 | 14 | 14 | 43 | 14 | **0** | **0** | **0** | - |

* The numbers in bold represent the intrapopulation polymorphism rates of the pure lines.

IRAP-PCR results for *Nikita* retrotransposon were presented in Fig. 3 and Fig. 4. Polymorphism rates were 0–50% for BAR and COL lines, 9–27% for RIR line and 0–56% for BRO lines. *Nikita* movements were detected in all lines (Fig. 3 and Table 3)

Table 3
Polymorphism rates for the first group in terms of *Nikita* retrotransposon. 1–4, BAR line samples; 5–8, COL line samples; 9–12, RIR line samples; 13–16, BRO line samples

| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | - | | | | | | | | | | | | | | | |
| 2 | **50** | - | | | | | | | | | | | | | | |
| 3 | **50** | **38** | - | | | | | | | | | | | | | |
| 4 | **50** | **38** | **0** | - | | | | | | | | | | | | |
| 5 | 50 | 33 | 38 | 38 | - | | | | | | | | | | | |
| 6 | 40 | 43 | 44 | 44 | **17** | - | | | | | | | | | | |
| 7 | 18 | 50 | 36 | 36 | **50** | **40** | - | | | | | | | | | |
| 8 | 40 | 43 | 44 | 44 | **17** | **0** | **40** | - | | | | | | | | |
| 9 | 18 | 50 | 36 | 36 | 50 | 40 | 18 | 40 | - | | | | | | | |
| 10 | 25 | 55 | 27 | 27 | 55 | 45 | 9 | 45 | **9** | - | | | | | | |
| 11 | 27 | 60 | 45 | 45 | 44 | 33 | 10 | 33 | **27** | **18** | - | | | | | |
| 12 | 27 | 44 | 30 | 30 | 44 | 33 | 10 | 33 | **27** | **18** | **20** | - | | | | |
| 13 | 60 | 50 | 50 | 50 | 20 | 33 | 60 | 33 | 60 | 64 | 56 | 56 | - | | | |
| 14 | 60 | 50 | 50 | 50 | 20 | 33 | 60 | 33 | 60 | 64 | 56 | 56 | **0** | - | | |
| 15 | 42 | 73 | 45 | 45 | 60 | 50 | 27 | 50 | 27 | 18 | 20 | 36 | **56** | **56** | - | |
| 16 | 27 | 60 | 45 | 45 | 44 | 33 | 27 | 33 | 10 | 18 | 20 | 36 | **56** | **56** | **20** | - |

* The numbers in bold represent the intrapopulation polymorphism rates of the pure lines.

Polymorphism ratios were 0–22% for BLA line and 0–30% for BLU line. Similar to *Copia-like* results, MAR lines showed no polymorphism for *Nikita* retrotransposon. Moreover, no polymorphism was detected in L-54 samples (Fig. 4 and Table 4).

Table 4
Polymorphism rates for the second group in terms of *Nikita* retrotransposon. 1–4, BLA line samples; 5–8, BLU line samples; 9–12, L-54 line samples; 13–16, MAR line samples

| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | - | | | | | | | | | | | | | | | |
| 2 | **13** | - | | | | | | | | | | | | | | |
| 3 | **22** | **13** | - | | | | | | | | | | | | | |
| 4 | **0** | **13** | **22** | - | | | | | | | | | | | | |
| 5 | 20 | 30 | 36 | 20 | - | | | | | | | | | | | |
| 6 | 30 | 40 | 45 | 30 | **10** | - | | | | | | | | | | |
| 7 | 13 | 25 | 33 | 13 | **30** | **22** | - | | | | | | | | | |
| 8 | 13 | 25 | 33 | 13 | **30** | **22** | **0** | - | | | | | | | | |
| 9 | 30 | 40 | 30 | 30 | 42 | 36 | 22 | 22 | - | | | | | | | |
| 10 | 30 | 40 | 30 | 30 | 42 | 36 | 22 | 22 | **0** | - | | | | | | |
| 11 | 30 | 40 | 30 | 30 | 42 | 36 | 22 | 22 | **0** | **0** | - | | | | | |
| 12 | 30 | 40 | 30 | 30 | 42 | 36 | 22 | 22 | **0** | **0** | **0** | - | | | | |
| 13 | 22 | 33 | 22 | 22 | 36 | 30 | 13 | 13 | 11 | 11 | 11 | 11 | - | | | |
| 14 | 22 | 33 | 22 | 22 | 36 | 30 | 13 | 13 | 11 | 11 | 11 | 11 | **0** | - | | |
| 15 | 22 | 33 | 22 | 22 | 36 | 30 | 13 | 13 | 11 | 11 | 11 | 11 | **0** | **0** | - | |
| 16 | 22 | 33 | 22 | 22 | 36 | 30 | 13 | 13 | 11 | 11 | 11 | 11 | **0** | **0** | **0** | - |

* The numbers in bold represent the intrapopulation polymorphism rates of the pure lines.

The movements of *SIRE* was detected in all samples (Fig. 5 and Fig. 6). The ratios were 9–25% for BAR line, 9–27% for COL line and 0–38% for RIR line and 15–36% for BRO line (Fig. 5 and Table 5)

Table 5
Polymorphism rates for the first group in terms of *SIRE* retrotransposon. 1–4, BAR line samples; 5–8, COL line samples; 9–12, RIR line samples; 13–16, BRO line samples

| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | - | | | | | | | | | | | | | | | |
| 2 | **9** | - | | | | | | | | | | | | | | |
| 3 | **18** | **9** | - | | | | | | | | | | | | | |
| 4 | **18** | **25** | **18** | - | | | | | | | | | | | | |
| 5 | 9 | 17 | 25 | 9 | - | | | | | | | | | | | |
| 6 | 20 | 27 | 20 | 20 | **27** | - | | | | | | | | | | |
| 7 | 31 | 23 | 31 | 31 | **23** | **33** | - | | | | | | | | | |
| 8 | 18 | 25 | 33 | 18 | **9** | **20** | **17** | - | | | | | | | | |
| 9 | 23 | 15 | 23 | 23 | 15 | 38 | 8 | 23 | - | | | | | | | |
| 10 | 17 | 8 | 17 | 17 | 8 | 33 | 15 | 17 | **8** | - | | | | | | |
| 11 | 50 | 42 | 36 | 36 | 42 | 55 | 46 | 50 | **38** | **33** | - | | | | | |
| 12 | 17 | 8 | 17 | 17 | 8 | 33 | 15 | 17 | **8** | **0** | **33** | - | | | | |
| 13 | 25 | 17 | 9 | 25 | 31 | 27 | 23 | 38 | 15 | 23 | 42 | 23 | - | | | |
| 14 | 10 | 18 | 10 | 10 | 18 | 11 | 38 | 27 | 31 | 25 | 45 | 25 | **18** | - | | |
| 15 | 23 | 15 | 23 | 23 | 15 | 38 | 8 | 23 | 0 | 8 | 38 | 8 | **15** | **31** | - | |
| 16 | 40 | 33 | 29 | 29 | 33 | 43 | 27 | 40 | 20 | 27 | 43 | 27 | **21** | **36** | **20** | - |

* The numbers in bold represent the intrapopulation polymorphism rates of the pure lines.

*SIRE* showed varying results BLA, BLU, L-54 and MAR lines. The highest ratios were observed in L-54 line (0–54%). On the other hand, BLA and BLU indicated low polymorphism rates (0–8%) (Fig. 6 and Table 6).

Table 6
Polymorphism rates for the second group in terms of *SIRE* retrotransposon. 1–4, BLA line samples; 5–8, BLU line samples; 9–12, L-54 line samples; 13–16, MAR line samples

| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | - | | | | | | | | | | | | | | | |
| 2 | **8** | - | | | | | | | | | | | | | | |
| 3 | **8** | **0** | - | | | | | | | | | | | | | |
| 4 | **0** | **8** | **8** | - | | | | | | | | | | | | |
| 5 | 8 | 0 | 0 | 8 | - | | | | | | | | | | | |
| 6 | 8 | 0 | 0 | 8 | **0** | - | | | | | | | | | | |
| 7 | 0 | 8 | 8 | 0 | **8** | **8** | - | | | | | | | | | |
| 8 | 8 | 0 | 0 | 8 | **0** | **0** | **8** | - | | | | | | | | |
| 9 | 50 | 54 | 54 | 50 | 54 | 54 | 50 | 54 | - | | | | | | | |
| 10 | 23 | 15 | 15 | 23 | 15 | 15 | 23 | 15 | **45** | - | | | | | | |
| 11 | 54 | 57 | 57 | 54 | 57 | 57 | 54 | 57 | **14** | **50** | - | | | | | |
| 12 | 23 | 15 | 15 | 23 | 15 | 15 | 23 | 15 | **45** | **0** | **50** | - | | | | |
| 13 | 46 | 50 | 50 | 46 | 50 | 50 | 46 | 50 | 44 | 42 | 50 | 42 | - | | | |
| 14 | 14 | 7 | 7 | 14 | 7 | 7 | 14 | 7 | 57 | 21 | 60 | 21 | **43** | - | | |
| 15 | 46 | 50 | 50 | 46 | 50 | 50 | 46 | 50 | 44 | 42 | 50 | 42 | **0** | **43** | - | |
| 16 | 14 | 7 | 7 | 14 | 7 | 7 | 14 | 7 | 57 | 21 | 60 | 21 | **43** | **0** | **43** | - |

* The numbers in bold represent the intrapopulation polymorphism rates of the pure lines.

*Sukkula* results were indicated in Fig. 7 and Fig. 8. Polymorphism rates were 0–22% for BAR, COL andRIR lines. Furthermore, there were 0–13% polymorphism ratios for BRO line (Fig. 7 and Table 7).

Table 7
Polymorphism rates for the first group in terms of *Sukkula* retrotransposon. 1–4, BAR line samples; 5–8, COL line samples; 9–12, RIR line samples; 13–16, BRO line samples

| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | - | | | | | | | | | | | | | | | |
| 2 | **0** | - | | | | | | | | | | | | | | |
| 3 | **0** | **0** | - | | | | | | | | | | | | | |
| 4 | **22** | **22** | **22** | - | | | | | | | | | | | | |
| 5 | 30 | 30 | 30 | 13 | - | | | | | | | | | | | |
| 6 | 11 | 11 | 11 | 13 | **22** | - | | | | | | | | | | |
| 7 | 11 | 11 | 11 | 13 | **22** | **0** | - | | | | | | | | | |
| 8 | 20 | 20 | 20 | 22 | **11** | **11** | **11** | - | | | | | | | | |
| 9 | 0 | 0 | 0 | 22 | 30 | 11 | 11 | 20 | - | | | | | | | |
| 10 | 0 | 0 | 0 | 22 | 30 | 11 | 11 | 20 | **0** | - | | | | | | |
| 11 | 11 | 11 | 11 | 13 | 22 | 0 | 0 | 11 | **11** | **11** | - | | | | | |
| 12 | 22 | 22 | 22 | 25 | 33 | 13 | 13 | 22 | **22** | **22** | **13** | - | | | | |
| 13 | 22 | 22 | 22 | 0 | 13 | 13 | 13 | 22 | 22 | 22 | 13 | 25 | - | | | |
| 14 | 22 | 22 | 22 | 0 | 13 | 13 | 13 | 22 | 22 | 22 | 13 | 25 | **0** | - | | |
| 15 | 22 | 22 | 22 | 0 | 13 | 13 | 13 | 22 | 22 | 22 | 13 | 25 | **0** | **0** | - | |
| 16 | 11 | 11 | 11 | 13 | 22 | 22 | 22 | 30 | 11 | 11 | 22 | 33 | **13** | **13** | **13** | - |

* The numbers in bold represent the intrapopulation polymorphism rates of the pure lines.

Polymorhism rates were increased in BLU line (0–38%). However, the results were 13% for BLA and 0–17% for L-54 lineNo polymorphism was detected for MAR line in terms of *Sukkula* retrotransposon (Fig. 8 and Table 8).

Table 8
Polymorphism rates for the second group in terms of *Sukkula* retrotransposon. 1–4, BLA line samples; 5–8, BLU line samples; 9–12, L-54 line samples; 13–16, MAR line samples

| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | - | | | | | | | | | | | | | | | |
| 2 | **13** | - | | | | | | | | | | | | | | |
| 3 | **13** | **0** | - | | | | | | | | | | | | | |
| 4 | **0** | **13** | **13** | - | | | | | | | | | | | | |
| 5 | 0 | 13 | 13 | 0 | - | | | | | | | | | | | |
| 6 | 0 | 13 | 13 | 0 | **0** | - | | | | | | | | | | |
| 7 | 38 | 29 | 29 | 38 | **38** | **38** | - | | | | | | | | | |
| 8 | 0 | 13 | 13 | 0 | **0** | **0** | **38** | - | | | | | | | | |
| 9 | 38 | 29 | 29 | 38 | 38 | 38 | 0 | 38 | - | | | | | | | |
| 10 | 25 | 14 | 14 | 25 | 25 | 25 | 17 | 25 | **17** | - | | | | | | |
| 11 | 38 | 29 | 29 | 38 | 38 | 38 | 0 | 38 | **0** | **17** | - | | | | | |
| 12 | 25 | 14 | 14 | 25 | 25 | 25 | 17 | 25 | **17** | **0** | **17** | - | | | | |
| 13 | 0 | 13 | 13 | 0 | 0 | 0 | 38 | 0 | 38 | 25 | 38 | 25 | - | | | |
| 14 | 0 | 13 | 13 | 0 | 0 | 0 | 38 | 0 | 38 | 25 | 38 | 25 | **0** | - | | |
| 15 | 0 | 13 | 13 | 0 | 0 | 0 | 38 | 0 | 38 | 25 | 38 | 25 | **0** | **0** | - | |
| 16 | 0 | 13 | 13 | 0 | 0 | 0 | 38 | 0 | 38 | 25 | 38 | 25 | **0** | **0** | **0** | - |

* The numbers in bold represent the intrapopulation polymorphism rates of the pure lines.

# Phylogenetic tree

The phylogenetic tree was constructed according to the Jaccard similarity index (Jaccard, 1908) by using GelJ version 2.0 software and represented in Fig. 9.

Pure lines were separated into two distinct groups by retrotransposon-based polymorphism. MAR belonged to a single clade which was basal to the branch containing other lines. Moreover, L-54 was a sister group to BLU while COL shared high sequence homology with BAR according to *Copia-like*, *Nikita*, *SIRE* and *Sukkula* retrotransposons.

# Discussion

Mutations are sources of genetic variation and epigenetic rearrangements can also support over environmental adaptation. Transposons are one of epigenetic mechanisms, affecting domestication, and exaptation processes. Interactions between transposons, the host genome, and even environmental factors influence changes in genome structure within a population. These interactions result in events that affect individuals and populations differently depending on their suitability to the environment (Capy, 2021; Mercan et al., 2022b).

TEs have exhibited different polymorphism rates between common and local breeds. For example, common breeds showed a more intensive band profile than the local Gerze chicken breed regarding *Nikita* and *Sukkula* retrotransposons. In our previous research, the frequency of genetic polymorphisms in the *Nikita* retrotransposon ranged between 0–60%. In contrast, no such polymorphisms were found in the *Sukkula* retrotransposon (Mercan et al., 2022a). Although TEs may have higher polymorphism ratios among individuals than breed lines (Serrato-Capuchina & Matute, 2018), polymorphism rates were detected higher between lines than among individuals in this study. *Copia-like*, *Nikita*, *SIRE*, and *Sukkula* retrotransposons indicated varying polymorphism rates amongst pure lines.

Coevolution between TEs and their hosts is a significant issue that shapes TE diversity and impacts the likelihood of insertions reaching high frequencies (Bourgeois & Boissinot, 2019). The *gag* genes of *Ty3/ Gypsy*, *Ty1/ Copia* and *Bel-Pao* involved in co-option events were detected in several animal, plant and fungal species. However, it has been found that the co-option of the long terminal repeat (LTR) retrotransposon *gag* genes during the early evolution of eukaryotes are rare (Wang & Han, 2021). Similarly, internal domains and LTR sequences of *SIRE* were investigated by sequencing. Partial *gag*, *rt* and *env* genes' sequences were detected in human genome by performing sequence and bioinformatic analyses. According to the bioinformatic analysis, partial *SIRE env* sequences were interestingly detected in human and chimpanzee chromosome 1 (Guner et al., 2022).

Early comparisons of the genomes of chickens and zebra finches suggested that bird genomes were stable compared to mammals. However, further research has revealed that the avian genome is quite dynamic when examined at a finer resolution. Many intrachromosomal rearrangements have been discovered across bird species, and interchromosomal recombination has been observed in falcons, parrots, and sandpipers.

Recent studies of Galbraith et al. (2021) indicate that similar patterns of TE family expansion are observed across amniotes and suggest mechanisms of TE-driven genome evolution can be generalised across tetrapods. Within birds, *chicken repeat 1* (*CR1*) retrotransposons constitute a large portion of the characteristic avian genome, nearly 7–10%. The MHC-Y (Major histocompatibility complex Y) gene region contains many TE sequences, mostly LTR retrotransposons. Like other avians, *CR1* retrotransposons constitute the most repeated transposons in the chicken genome. *CR1* retrotransposons are more abundant in the chicken genome than in the MHC-Y gene region. An idea about a possible relation between TEs and the evolution of the MHC-Y gene region. This idea suggests that the diversity in this

gene region can be advantageous in populations subject to various diseases (Galbraith et al., 2021; Goto et al., 2022).

Retrotransposons occasionally produce different allele copies because they arbitrarily bind the genome. Allele copies and mutations may improve certain traits such as the double muscling gene in Belgian Blue cattle breed. The Belgian Blue is known for its extreme degree of muscling with muscle hypertrophy, mainly caused by a loss-of-function mutation in the *myostatin* (*MSTN*) gene. In this gene, there is a deletion called *nt821* (*del11*) with 11 bp in length, causing a premature stop-codon and a dysfunctional protein. Besides, arbitrary bindings are problematic for IRAP-PCR polymorphism. Retrotransposon-based molecular markers have serious flaws. They need sequence data for designing specific primers. All retrotransposon-based molecular marker techniques are required to know the LTR sequence to design retrotransposon-specific primers. In distant species, LTRs do not contain conserved motifs. Because of this, we cannot directly amplify them in PCR, and primers will not work in every PCR procedure. Still, rapid retrotransposon isolation methods based on PCR with conserved primers have been designed for retrotransposable elements. However, it may still be necessary to clone and sequence hundreds of clones to get just a few good primer sequences. The genome does not always have many retrotransposon or primer targets that can be too wide apart, leading to unsuccessful amplification in PCR. Therefore, the IRAP method is not suitable for DNA fingerprinting. Thus, further analyses are required to confirm (Kalendar et al., 2021; Meyermans et al., 2022).

## Conclusion

Because TEs can be activated after a change in environmental conditions, they become a source of epigenetic variation. Their movements cause beneficial or detrimental effects for organisms- based on situations. TEs allow the maintenance of active copies unless the epigenetic marks are accompanied by a more significant TE variability that immobilises them. Besides, horizontally transferred transposons produce copies at different rates on various breed lines. These phenomena may be a key to explaining why crossbreds have higher yields than pure lines.

## Declarations

# CONFLICT OF INTEREST

The authors have no relevant financial or non-financial interests to disclose.

## References

1. Bourgeois Y, Boissinot S (2019) On the population dynamics of junk: a review on the population genomics of transposable elements. Genes 10(6):419. https://doi.org/10.3390/genes10060419

2. Capy P (2021) Taming, domestication and exaptation: Trajectories of transposable elements in genomes. Cells 10(12):3590. https://doi.org/10.3390/cells10123590
3. Chesnay C, Kumar A, Pearce S (2007) Genetic diversity of *SIRE-1* retroelements in annual and perennial Glycine species revealed using SSAP. Cell Mol Biology Lett 12(1):103–110. https://doi.org/10.2478/s11658-006-0054-y
4. Galbraith JD, Kortschak RD, Suh A, Adelson DL (2021) Genome stability is in the eye of the beholder: CR1 retrotransposon activity varies significantly across avian diversity. Genome Biol Evol 13(12):evab259. https://doi.org/10.1093/gbe/evab259
5. Goto RM, Warden CD, Shiina T, Hosomichi K, Zhang J, Kang TH, Miller MM (2022) The *Gallus gallus* RJF reference genome reveals an MHCY haplotype organized in gene blocks that contain 107 loci including 45 specialized, polymorphic MHC class I loci, 41 C-type lectin-like loci, and other loci amid hundreds of transposable elements. *G3, 12*(11), jkac218. doi:https://doi.org/10.1093/g3journal/jkac218
6. Göger H, Demirtaş ŞE, Yurtoğullari Ş, Taşdemir AN, Şenkal UE, Boyali B (2017) Breeding studies on pure lines at poultry research institute. J Poult Res 14(2):30–38
7. Guner BC, Karlik E, Gozukirmizi N (2022) Remnants of *SIRE1* retrotransposons in human genome? J Genet 102(1):10. https://doi.org/10.1007/s12041-022-01398-3
8. Heras J, Domínguez C, Mata E, Pascual V, Lozano C, Torres C, Zarazaga M (2015) GelJ–a tool for analyzing DNA fingerprint gel images. BMC Bioinformatics 16(1):1–8. https://doi.org/10.1186/s12859-015-0703-0
9. Jaccard P (1908) Nouvelles recherches sur la distribution florale. Bull Soc Vaud Sci Nat 44:223–270. https://doi.org/10.5169/seals-268384
10. Kalendar R, Muterko A, Boronnikova S (2021) Retrotransposable elements: DNA fingerprinting and the assessment of genetic diversity. Mol Plant Taxonomy: Methods Protocols 263–286. https://doi.org/10.1007/978-1-0716-0997-2_15
11. Kalendar R, Schulman AH (2006) IRAP and REMAP for retrotransposon-based genotyping and fingerprinting. Nat Protoc 1(5):2478–2484. https://doi.org/10.1038/nprot.2006.377
12. Kim WR, Park EG, Lee YJ, Bae WH, Lee DH, Kim H-S (2022) Integration of TE Induces Cancer Specific Alternative Splicing Events. Int J Mol Sci 23(18):10918. https://doi.org/10.3390/ijms231810918
13. Leigh F, Kalendar R, Lea V, Lee D, Donini P, Schulman A (2003) Comparison of the utility of barley retrotransposon families for genetic analysis by molecular marker techniques. Mol Genet Genomics 269:464–474. https://doi.org/10.1007/s00438-003-0850-2
14. Mercan L, Bulbul CE, Bilgi F, Marakli S (2022a) Determination of plant-specific retrotransposons in chicken. Turkish J Veterinary Anim Sci 46(1):67–73. https://doi.org/10.3906/vet-2102-56
15. Mercan L, Bulbul CE, Marakli S (2022b) Genetic diversity among queen bee, worker bees and larvae in terms of retrotransposon movements. Genet Resour Crop Evol 69(4):1671–1683. https://doi.org/10.1007/s10722-021-01331-0

16. Meyermans R, Janssens S, Coussé A, Gorssen W, Hubin X, Mayeres P, Buys N (2022) Myostatin mutation causing double muscling could affect increased psoroptic mange sensitivity in dual purpose Belgian Blue cattle. Animal 16(3):100460. https://doi.org/10.1016/j.animal.2022.100460
17. Miller S, Dykes D, Polesky H (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic acids res 16(3):1215
18. Peters J, Lebrasseur O, Irving-Pease EK, Paxinos PD, Best J, Smallman R, Frantz L (2022) The biocultural origins and dispersal of domestic chickens. *Proceedings of the National Academy of Sciences, 119*(24), e2121978119. doi:https://doi.org/10.1073/pnas.2121978119
19. Serrato-Capuchina A, Matute DR (2018) The role of transposable elements in speciation. Genes 9(5):254. https://doi.org/10.3390/genes9050254
20. Wang J, Han G-Z (2021) Unearthing LTR retrotransposon *gag* genes co-opted in the deep evolution of eukaryotes. Mol Biol Evol 38(8):3267–3278. https://doi.org/10.1093/molbev/msab101

# Figures

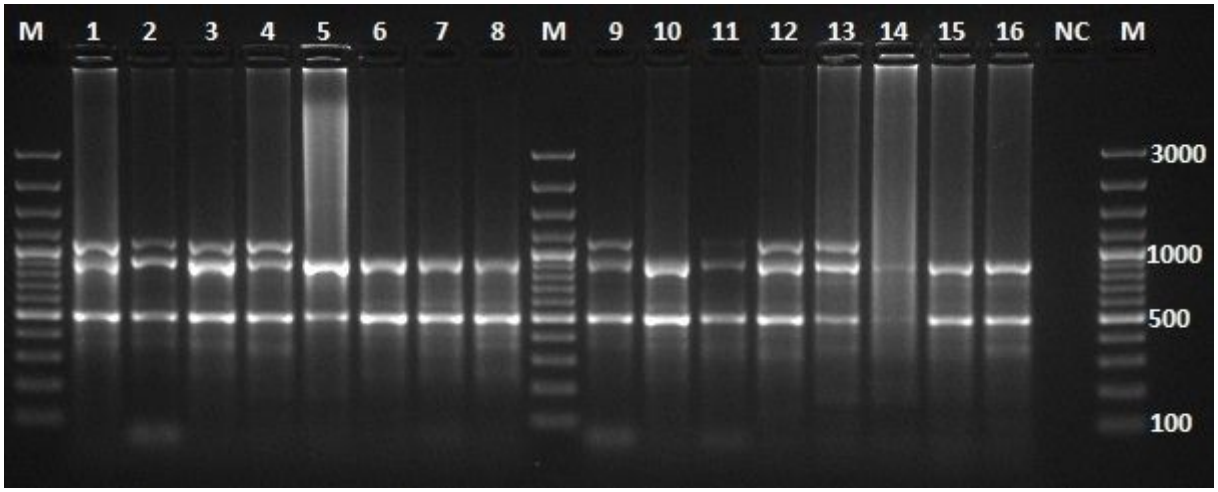
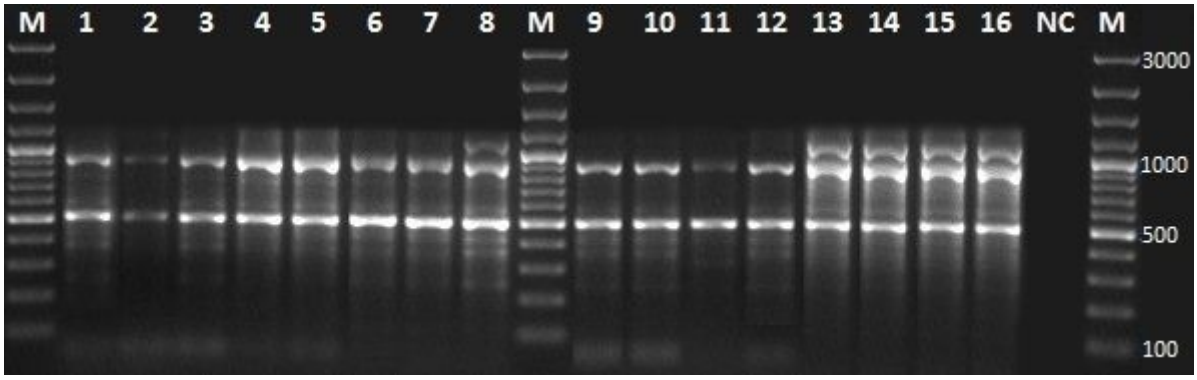


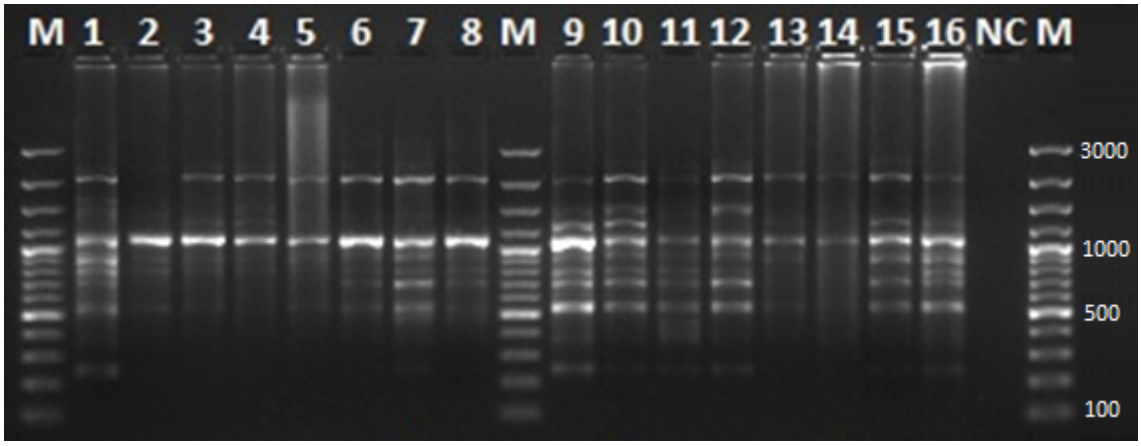

**Figure 1**

IRAP-PCR result for the first group in terms of *Copia-like* retrotransposon. M, marker; NC, negative control; 1-4, BAR line samples; 5-8, COL line samples; 9-12, RIR line samples; 13-16, BRO line samples



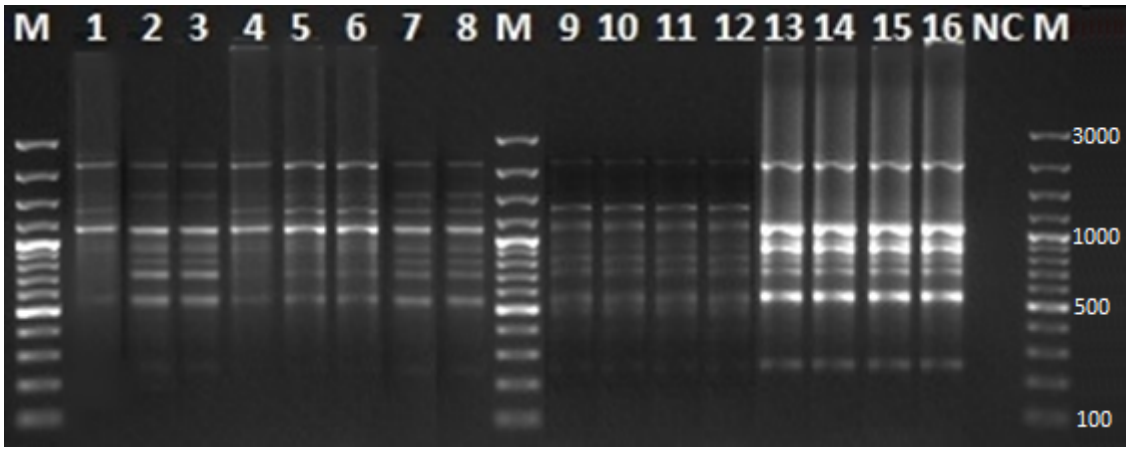
## Figure 2

IRAP-PCR result for the second group in terms of *Copia-like* retrotransposon. M, marker; NC, negative control; 1-4, BLA line samples; 5-8, BLU line samples; 9-12, L-54 line samples; 13-16, MAR line samples




## Figure 3

IRAP-PCR result for the first group in terms of *Nikita* retrotransposon. M, marker; NC, negative control; 1-4, BAR line samples; 5-8, COL line samples; 9-12, RIR line samples; 13-16, BRO line samples




## Figure 4

IRAP-PCR result for the second group in terms of *Nikita* retrotransposon. M, marker; NC, negative control; 1-4, BLA line samples; 5-8, BLU line samples; 9-12, L-54 line samples; 13-16, MAR line samples

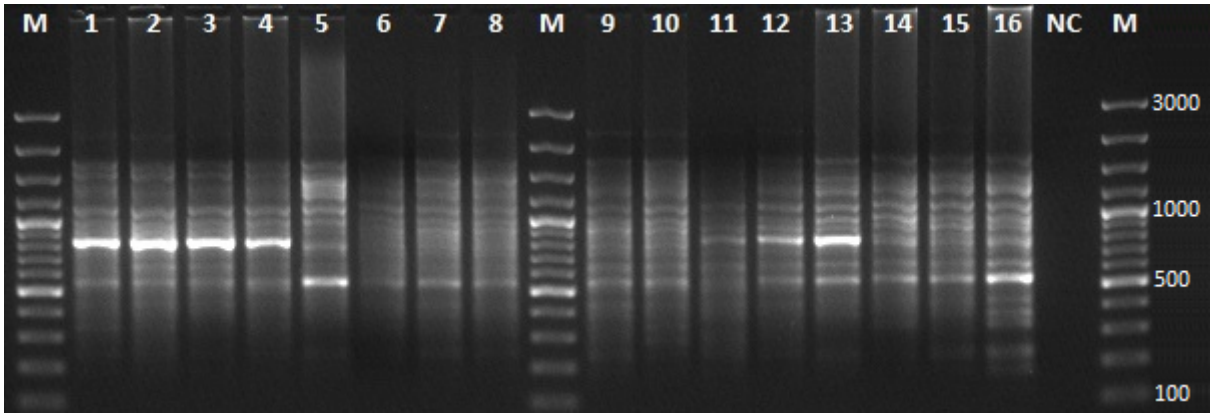



**Figure 5**

IRAP-PCR result for the first group in terms of *SIRE* retrotransposon. M, marker; NC, negative control; 1-4, BAR line samples; 5-8, COL line samples; 9-12, RIR line samples; 13-16, BRO line samples

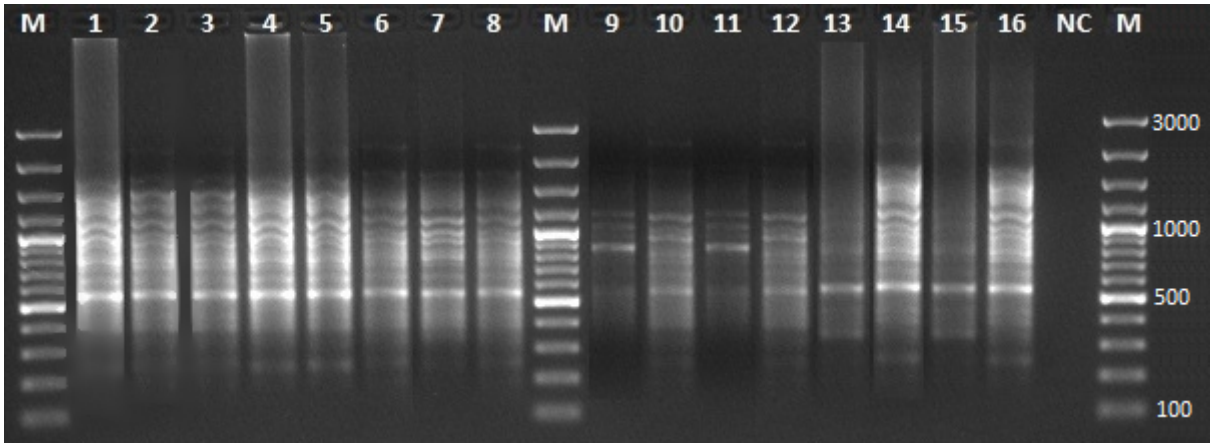



**Figure 6**

IRAP-PCR result for the second group in terms of *SIRE* retrotransposon. M, marker; NC, negative control; 1-4, BLA line samples; 5-8, BLU line samples; 9-12, L-54 line samples; 13-16, MAR line samples

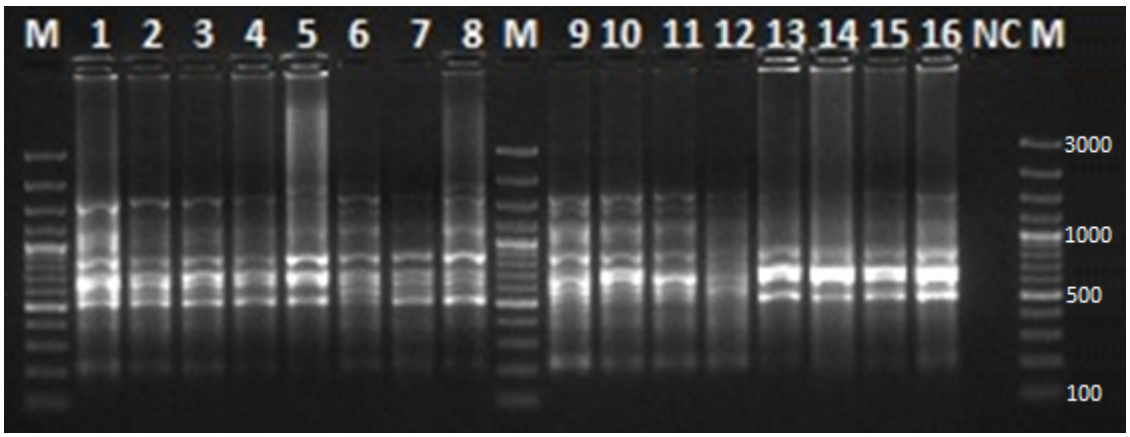



**Figure 7**

IRAP-PCR result for the first group in terms of *Sukkula* retrotransposon. M, marker; NC, negative control; 1-4, BAR line samples; 5-8, COL line samples; 9-12, RIR line samples; 13-16, BRO line samples

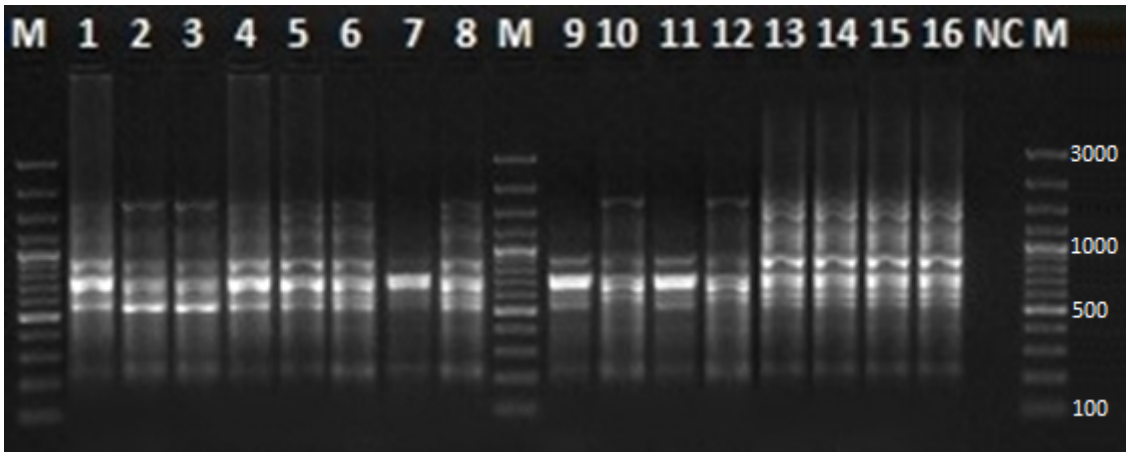
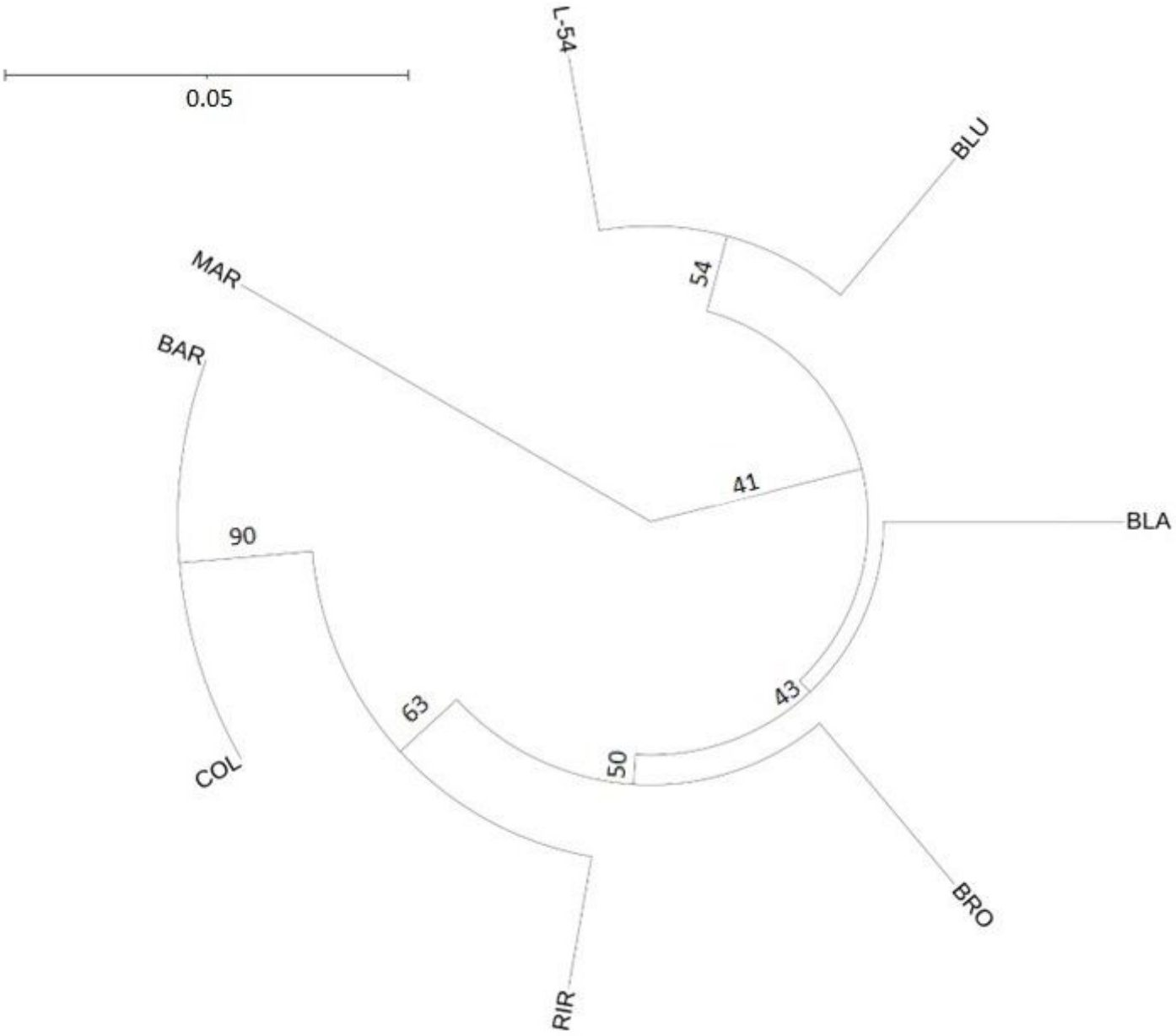



**Figure 8**

IRAP-PCR result for the second group in terms of *Sukkula* retrotransposon. M, marker; NC, negative control; 1-4, BLA line samples; 5-8, BLU line samples; 9-12, L-54 line samples; 13-16, MAR line samples




## Figure 9

Phylogenetic dendrogram of eight pure line chickens in terms of retrotransposons' polymorphisms