RETROVIRUSES REVEAL THE ANCIENT HISTORY OF

JAPANESE BREEDS AND BROILERS

September 2025 - Text: Sigrid van Dort

...and the jungle fowls that went into them...

A groundbreaking study published last april 2025 has used an unconventional genetic tool to trace the ancestry of Japanese chickens: ancient viral DNA frozen in their genomes.

When retroviruses infect an animal's reproductive cells, they sometimes become permanent passengers in the DNA, passed down through generations like any other gene. These 'endogenous retroviruses' (ERVs) are essentially viral fossils, and because they stay in fixed locations on chromosomes, they work remarkably well as genetic markers*). One of the most 'famous' ERVs, every breeder knows, is recessive white. Different populations carry different ERVs, making them useful for tracing historical relationships going very far back in the past (thousands of years!).

Researchers from Nippon Veterinary and Life Science University in Japan analysed the genomes of commercial Ross 308 broilers and also two Japanese breeds: Tosa-Jidori (considered one of Japan's oldest native breeds with over 2,000 years of history) and Yakido (a tall Malay type breed with distinctive black feathers). They identified 172 ERVs across the three chicken breeds.

Broilers carried 119 ERV genes, whilst Tosa-Jidori had 80 and Yakido 86. There were 28 sites in common amongst all three chickens.

Broilers showed a higher genetic diversity, their heterozygosity was 0.28 compared to 0.17 and 0.19 for the original Japanese breeds, which reflects the broiler's crossbred origins.

The original Japanese breeds showed more homozygous ERV genes (10 in Tosa-Jidori, 14 in Yakido), showing their more isolated breeding histories.

What are ERVs? When ancient viruses infected the wild ancestors of chickens, sometimes the viral DNA got stuck in an egg or sperm cell. That viral DNA was then passed down through generations, like any other gene... permanently. That viral DNA got copied and passed down to all the descendants, generation after generation. These ancient viral leftovers are called ERVs (endogenous retroviruses). They sit in specific spots on the

chromosomes, like
bookmarks on different
pages. Most have broken
down over time and can't
work as viruses
anymore, but they're still
there.

Different chicken breeds inherited different ERV bookmarks. Scientists can look at which spots have ERVs and which don't, it's like comparing fingerprints to

figure out which breeds are closely related and which evolved separately.

About 5-8% of your DNA is actually old virus leftovers. Chickens have them too, obviously, and each pattern tells part of their evolutionary history.



Tosa-Jidori by Masaoki Tsudzuki



Ross 308 broiler.



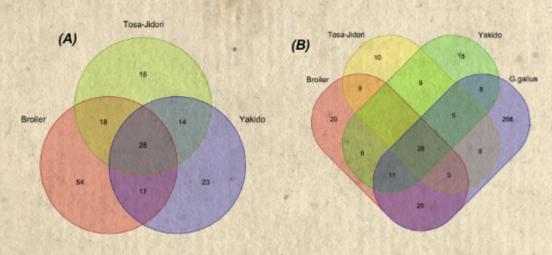
Yakido (Mie game) by DOI:10.1016/j.psj.2021.101582

Jungle fowl ancestors...

When the team compared their results with previously published junglefowl genomes, they found that 94 of the ERV genes matched those of red junglefowl (*Gallus gallus*). Red jungle fowl is long recognised as the primary ancestor of our domestic chickens. However, one locus was shared specifically with grey junglefowl (*G. sonneratii*)

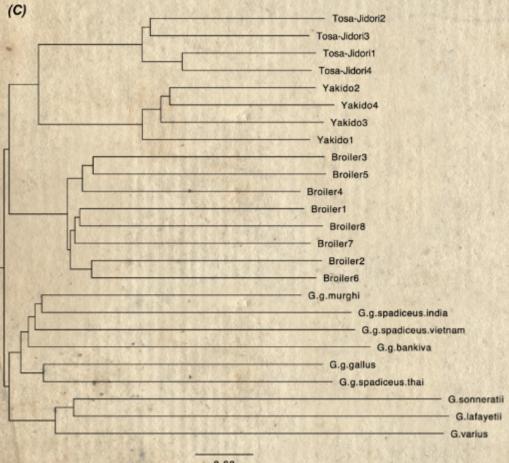
which was absent in red junglefowl, a direct evidence of genetic contribution from **multiple** wild species.

The phylogenetic analysis**), which clustered chickens based on ERV presence or absence, placed all three domestic breeds close to red junglefowl



subspecies from India, Vietnam, and Thailand, whilst grey junglefowl, Sri Lankan, and green junglefowl formed more distant branches. *This supports the hypothesis that domestic chickens have a complex ancestry involving multiple Gallus species*.

- **Fig. 1.** Number of endogenous retrovirus (ERV) loci detected among broiler, Tosa-Jidori, Yakido and gallus genus as well as phylogenetic trees.
- (A) Number of ERV loci across broiler, Tosa-Jidori, and Yakido and the overlap between each ERV locus.
- (B) Number of ERV loci across broiler, Tosa-Jidori, Yakido, and red junglefowl and the overlap between each ERV locus.
- **(C)** Phylogenetic tree constructed based on the presence or absence of ERV loci. The scale bar indicates the distance calculated from the presence/absence matrix.



Intriguingly, 77 of the 172 ERV genes are new, so not previously reported in junglefowl or in chicken breeds. Some of these insertions weren't just neutral genetic passengers: the researchers found that ERVs near two genes (NAALAD2 and PAICS) significantly altered their expression levels, with the viral DNA sequences apparently acting as regulatory elements***).

For breeders of rare and heritage chickens, this research underscores the genetic uniqueness of ancient indigenous breeds. Each population has its own unique signs of ancient viral infections, changes to chromosomes, and contributions from its **Table 1**. Comparison of observed and expected heterozygosity in different chickens.

Chicken	Observed	Expected
Broiler	0.28	0.23
Tosa-Jidori	0.17	0.14
Yakido	0.19	0.15

ancestors. As the authors explain, 'understanding how ERV insertions affect gene expression can help with future genetic research and breeding programmes for poultry that aim to improve health and productivity.'

These viral fossils are proving to be unexpectedly useful archaeologists, revealing chapters of chicken domestication that conventional genetic markers might miss entirely.





EXPLANATIONS OF THE TERMS MARKED WITH A STAR *)

*) **Genetic markers** are identifiable DNA differences that scientists use like signposts or labels to tell individuals, breeds, or populations apart. Think of them like postcode patterns, they tell you where someone lives without affecting who they are.



Common types of genetic markers are:

- Microsatellites short repeated DNA sequences that vary in length
- SNPs (single nucleotide polymorphisms) single-letter differences in DNA
- ERVs (what this paper uses) presence or absence of viral insertions at specific locations

Why care about genetics markers?

They let you: verify parentage or breed purity, trace ancestry and relationships between breeds, map out genetic diversity, and sometimes track traits (if a marker happens to sit near a gene that affects something visible)

So....

Most genetic markers are just 'differences', they don't necessarily *DO* anything themselves, but because they're inherited, they're useful for tracking the history of lineages. It's like using postcodes to follow a travelscheme, when you send a postcard from each place you visit; the postcode from that place doesn't make you different, it tells you only where you've been. In this study, the ERVs work perfectly as markers because they're stable, inherited, and each breed has its own unique pattern of where they occur in the genome.

**) Phylogenetic analysis for chicken breeders: a family tree based on genetics

Think of phylogenetic analysis as a family tree, but instead of using birth certificates and marriage records, scientists use DNA.

The basic idea

Phylogenetic analysis looks at what different breeds or species have in common genetically. The more genetic features they share, the more closely related they are.

How it works with retroviruses

In this study, the researchers looked at 172 different places in the genome where ancient viruses had inserted themselves. For each chicken, they recorded: "Does it have an ERV at location no. 1? Yes or no. Location no. 2? Yes or no..." and so on, creating a pattern like a genetic barcode.

Then they compared all the chickens:

- Ross 308 broiler has ERVs at locations 5, 12, 47, 89...
- Tosa-Jidori has ERVs at locations 12, 33, 47, 91...
- Red junglefowl has ERVs at locations 12, 47, 55, 91...

Reading the phylogenetic tree:

When they drew the phylogenetic tree (Figure **1C**), chickens that share many ERV locations cluster close together on branches. Chickens that share fewer locations sit on more distant branches.

What the tree showed:

- All three domestic types (broilers, Tosa-Jidori, Yakido) grouped near the red junglefowl branches, confirming red junglefowl as the primary ancestor.
- The other junglefowl species (grey, Sri Lankan, green) sat on more distant branches.
- Within the domestic chickens, each type formed its own sub-cluster, showing they're related but distinct populations.

Why this matters to breeders

It's proof of genetic distinctiveness. When Tosa-Jidori clusters separately from broilers, it's not just that they *look* different, they carry genuinely unique genetic signatures that have been preserved over centuries of breeding. This makes conservation of rare breeds scientifically important, not just sentimentally important.

It's like discovering that your rare breed isn't just a pretty variant of a common breed, it's a distinct genetic lineage with its own evolutionary story.

***) What ERVs as regulatory elements means...

Genes are like instruction manuals for making proteins, but they need control switches to determine *when*, *where*, and *how much* protein gets made. These control switches are called 'regulatory elements.'

What happened with the ERVs?

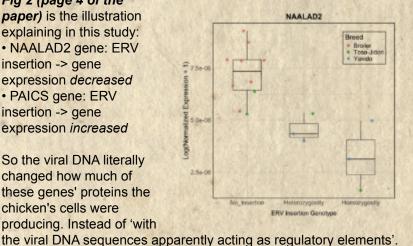
When these ancient viruses inserted themselves into the chicken genome, they didn't just sit there doing nothing. The viral DNA contained sequences that act like genetic switches, specifically, parts called LTRs (long terminal repeats) that can function as promoters or enhancers.

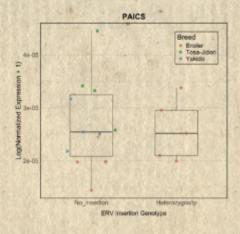
Fig 2 (page 4 of the paper) is the illustration explaining in this study:

 NAALAD2 gene: ERV insertion -> gene expression decreased

 PAICS gene: ERV insertion -> gene expression increased

So the viral DNA literally changed how much of these genes' proteins the chicken's cells were producing. Instead of 'with





You could also say:

- · 'with the viral DNA acting as genetic switches that turned genes up or down';
- 'with the viral DNA changing how active certain genes were';
- 'with the viral DNA altering gene expression levels';
- 'affecting how much protein these genes produced'.

The key point for breeders: these aren't just neutral genetic markers, some of them actually do something, potentially affecting the chicken's biology.

NERDS may ask:

What is PAICS?

Answer: phosphoribosylaminoimidazolesuccinocarboxamide synthase <- say that word out loud twice (this is biochemistry).

Nerds: AICS is a bifunctional enzyme that catalyses steps 6 and 7 of the de novo purine biosynthesis pathway (DNA/RNA building blocks). It's actually describing the exact chemical reaction the enzyme performs, it's synthesising a molecule called phosphoribosylaminoimidazolesuccinocarboxamide (also mercifully shortened to SAICAR). The name literally tells you what goes in and what comes out of the reaction, which is standard biochemical nomenclature, but it does get rather unwieldy.

What is does? (sigh....)

The enzyme has two distinct activities (after looking this up!)

- 1. Phosphoribosylaminoimidazole carboxylase (AIRC) located in the N-terminal
- 2. Phosphoribosylaminoimidazolesuccinocarboxamide synthase (SAICAR synthase) - located in the C-terminal region.

The enzymatic activities of these two functions copurify from chicken livers confirming that in chickens (as in other vertebrates), this is a single bifunctional protein rather than two separate enzymes. The chicken PAICS gene is located on chromosome 4 and encodes multiple protein isoforms through alternative splicing. This gene is essential for purine nucleotide synthesis, which is critical for DNA and RNA production, making it fundamental for cell growth and division in chickens. I did not find this out myself as you might understand, one can look things up...

Now you want to know what NAALAD2 gene with the ERV insertion does too. NAALAD2 is an enzyme involved in breaking down signalling molecules. No explanation is given why the expression is going down in broilers, nothing is said about the consequences. End of story in this research paper.

Article based on: Endogenous retrovirus loci and induced changes in gene expression in Japanese indigenous chickens, https://doi.org/10.1038/s41598-025-96881-z

If you want to learn more about the origin of the Tosa-Jidori: https://www.

animbiosci.org/ journal/view.php? doi=10.5713/ ajas.19.0932

On native **Japanese**

chickens: https:// www.angrin.tlri.gov. tw/%5C/apec2003/ Chapter3JpChicken .pdf

On Yakido, a research paper on egg yolk and albumen: Genotype affects free amino acids of egg yolk and albumen in Japanese indigenous breeds and commercial Brown layer chicken https://www. researchgate.net/ publication/ 355987914 Genotype affects free amino acids of egg yolk and albumen in <u>Japanese</u> indigenous breeds and commercial Brown layer chickens