**Allelic frequency of major genes in indigenous chickens of Kerala, India**

### ****Abstract****

Indigenous chickens in India represent a vital genetic resource due to their adaptability, phenotypic diversity, and contribution to rural livelihoods. This study aimed to evaluate the phenotypic distribution and allelic frequency of major genes affecting qualitative traits in indigenous chicken populations from two districts in Kerala. A total of 200 adult birds from Kozhikode and Kannur were assessed for plumage pattern, comb type, skin colour, and feather traits, including frizzling, naked neck, ptilopody, and crest. Phenotypic frequencies and corresponding allele distributions were calculated and tested against the hypothesis of dominant and recessive alleles were at equal frequency (p = q = 0.50). Results revealed considerable plumage variability, with multicolour types predominating, likely reflecting both cultural preferences and adaptive advantages. Traits governed by autosomal incompletely dominant genes such as pea comb, naked neck, frizzling, ptilopody, and crest were observed at low frequencies. Dominant alleles for frizzling (*F* = 0.005), naked neck (*Na* = 0.005), ptilopody (*Pti* = 0.008) and crest (*Cr* = 0.01) exhibited significant (P < 0.001) deviation from expected ratios, suggesting strong negative selection. Skin colour variation revealed a higher frequency of the recessive yellow allele (*w* = 0.60), consistent with ancestral hybridization between red and grey junglefowl. These findings indicate an ongoing loss of adaptive and aesthetic traits in indigenous chickens, underscoring the need for targeted conservation strategies to preserve their unique genetic heritage.

**Keywords**: Indigenous chicken, India, Qualitative traits, gene and genotype frequencies

**1. INTRODUCTION**

Domestic chickens (*Gallus gallus domesticus*) belong to the genus Gallus, which comprises four wild junglefowl species: the green junglefowl (*G. varius*), Ceylon junglefowl (*G. lafayettii*), grey junglefowl (*G. sonneratii*), and red junglefowl (*G. gallus*) (Clark and Johnsgard, 2001). While the red junglefowl was historically considered the sole progenitor of the modern chicken, recent genomic studies suggest a more complex domestication process involving interspecies hybridization between *G. gallus* and *G. sonneratii*, and to a lesser extent, *G. lafayettii* (Nishibori *et al*., 2005). The grey junglefowl, G*. sonneratii*, is endemic to peninsular India, particularly the states of Karnataka, Tamil Nadu, and Kerala. Consequently, indigenous chicken ecotypes from this region may have originated through extensive bidirectional introgression of *G. gallus* with *G. sonneratii* (Lawal *et al*., 2020).

Indigenous chickens play a vital role in rural food and livelihood security, contributing 30–80% of the total poultry population in many developing countries (Sati *et al*., 2022; Churchil, 2022). They are predominantly reared under scavenging or extensive systems with minimal inputs for housing, feeding, or healthcare (Kumar *et al*., 2013). Indigenous chickens display extensive phenotypic variation in qualitative traits such as plumage pattern, comb type, shank colour, and eye and beak pigmentation, significantly more than commercial breeds (Churchil *et al*., 2019; Maharani *et al*., 2021).

Indigenous chickens possess diverse variations on the structure, distribution and length of feathers, which contribute to their adaptability across various ecological zones and climatic conditions. They are well adapted to local conditions, including high predator pressure, tropical climates, and open environments with prevalent disease challenges (Kumar *et al*., 2016; Kumar and Churchil, 2025). According to Horst (1989), indigenous chicken ecotypes serve as valuable genetic reservoirs, especially for traits associated with tropical adaptability. Gowe and Fairfull (1995) identified climate as a major limiting factor in poultry production, noting that high ambient temperatures coupled with elevated humidity levels induce considerable stress in birds, thereby negatively impacting their productivity. Whole-genome analyses have shown that indigenous chickens harbour a higher proportion of rare single-nucleotide polymorphisms (SNPs) compared to commercial lines and even wild red junglefowl, confirming their status as rich reservoirs of functionally important, low-frequency genetic variants (Wu *et al*., 2024).

This diversity underscores their value in genetic conservation and future breeding programmes. Despite their importance, limited research has been conducted on the allele frequencies of major genes such as naked neck (Na), frizzle (F), polydactyly (Po), and ptylopody (Fsh) in indigenous chickens (Horst, 1988; Fayeye *et al*., 2006). Among these, the Na and F genes, responsible for altered distribution and structure of feather have been associated with improved heat tolerance (Horst, 1988). Marthur and Horst (1990) further reported that these genes, individually and in combination, influence growth and egg production traits. This highlights the need to characterize indigenous chickens across agroecological zones, not only to inventory and monitor these genetic resources but also to ensure their sustainable utilization under present and future challenges such as climate change. The present study aims to assess the genotype frequency of selected qualitative traits and the associated allelic frequency of major genes in indigenous chicken populations of Kerala.

**2. MATERIALS AND METHODS**

A field study was undertaken to assess the allelic frequencies of selected major genes in indigenous chickens by recording their phenotypic traits. A total of 200 birds were evaluated; 100 each from Kozhikode and Kannur districts in Kerala. Thrippangottur Panchayat in Kannur and Chekkiad Panchayat in Kozhikode were selected based on preliminary surveys that identified as these areas harbouring relatively pure populations of native chickens. This was attributed to their geographic remoteness, the absence of exotic germplasm introduction, and phenotypic traits consistent with traditional native chicken populations. Both Kozhikode and Kannur lie within the Northern Midlands agro-ecological zone, characterized by a humid tropical climate, low elevations of below 500 m above sea level and form part of the West Coast plains and Ghat region.

Each bird was individually examined for a set of qualitative phenotypic traits associated with major genes as per FAO (2012) guidelines. Traits recorded included plumage colour and pattern, comb type, skin colour and structure distribution and length of feather. Plumage pattern was categorized as Columbian, solid black, birchen, wild, brown, wheaten and multicolour (non-specific). Due to the phenotypic similarity among males with wheaten, wild, and brown genotypes, these were grouped under the “wild” phenotype. The other major genes of interest included pea comb (*P/p*), skin colour (*W/w*), feather structure such as frizzling (*F/f*), feather distribution like naked neck (*Na/na*) and ptilopody or feathered shank (*Pti/pti*), as well as feather length variation on the head, resulting in a crested head (*Cr/cr*).

 The frequency of each qualitative trait was expressed as a percentage of the total population as below.

$$Phenotypic frequency=\frac{Number of individuals carrying trait}{Total number of individuals sampled} X 100$$

Allelic frequencies were calculated from phenotypic observations using the Hardy–Weinberg principle, as described by Falconer and Mackay (1989), with the following formulae:

$q=\sqrt{\frac{m}{t}}$ ; and *p* = 1 - q

Where, *q* is the frequency of the recessive allele, *p* is the frequency of the dominant allele, *m* is the number of birds expressing the recessive phenotype and *t* is the total number of birds examined.

The frequencies of dominant and recessive alleles were assumed to be in a 1:1 ratio for all traits, except for frizzling, where a 1:2 ratio was considered due to the absence of lethal homozygous dominant individuals under the assumption of Hardy–Weinberg equilibrium.

The observed phenotypic frequencies were tested against the expected Mendelian ratios under the assumption of Hardy–Weinberg equilibrium: 3:1 for completely dominant traits such as skin colour and Columbian restriction of plumage pattern and 1:2:1 for incompletely dominant traits such as comb type, naked neck, ptilopody and crest. For the frizzling trait, which is lethal in the homozygous dominant state (FF), an adjusted phenotypic ratio of 0:2:1 was applied.

These assumptions were made within the framework of Hardy–Weinberg equilibrium, assuming equal frequencies of dominant and recessive alleles (p = q = 0.5), and the absence of evolutionary influences such as selection, mutation, migration, or genetic drift. Deviations of phenotype and allelic frequencies from this expected distribution were evaluated using the Chi-square (χ²) test:

$$χ^{2}=\frac{\sum\_{}^{}(observed - expeted)^{2}}{expected}$$

**3. RESULTS AND DISCUSSION**

**3.1 Plumage pattern**

The *E* locus in chickens, also known as the ‘Extension’ locus, is a multiallelic major gene that plays a crucial role in determining feather colour and pattern. This locus encodes the melanocortin 1 receptor (MC1R) and includes several key alleles such as *E* (extended black), *Eᴿ* (birchen), *e⁺* (wild-type), *eʷʰ* (wheaten), and *eᵇ* (brown) (Guo *et al*., 2010). In the present study, the estimated phenotypic frequencies among the indigenous chicken population of Kerala were: 0.27% solid black (*E*), 0.15% birchen (*Eᴿ*), 0.21% wild-type (*e⁺*), 0.04% brown (*eᵇ*), and 0.33% wheaten (*eʷʰ*). The occurrence of multiple E locus alleles in indigenous chickens in other parts of world has also been documented previously (Larivière and Leroy, 2010; Dávila *et al*., 2014). This allelic diversity likely reflects both the cultural preference for multicoloured plumage among local communities and the adaptive advantage of such plumage, which enhances camouflage in natural environments, aiding in predator evasion. Another important locus influencing plumage pattern is the Columbian restriction (*Co*), an incompletely dominant gene responsible for restricting black pigmentation to specific regions such as the hackle and tail in both sexes. In the present study, the frequency of the dominant *Co* allele was significantly lower (P < 0.001) than that of its recessive counterpart (*co⁺*), suggesting a possible selection pressure against the dominant allele (*Co*) by farmers (Table). This may be due to a preference for multicoloured birds, as *co+* allele permits broader expression of the diverse *E* locus alleles, thereby contributing to the maintenance of plumage colour variability in the indigenous chicken population.

**3.2 Comb type**

 Only single and pea comb types were observed, with pea combs present in 5% of birds (Table). All observed pea combs had a prominent middle ridge, suggesting a heterozygous genotype for this incompletely dominant trait. The high prevalence of single comb is in agreement with the earlier reports from tropical countries (Bhuiyan *et al*., 2005; Melesse and Negesse, 2011; Agarwal *et al*., 2020). The gene frequency of dominant and recessive alleles was hugely (P<0.001) imbalanced indicating, probably the natural selection that favours single combed birds as this helps in heat dissipation as an adaptive physiology in tropical climates. Beyond environmental adaptation, comb preferences also exhibit cultural variations; while single combs are widely favoured in many regions including India, rose combs are preferred in specific areas such as parts of Ethiopia (Chebo *et al*., 2023).

**3.3 Skin colour**

Although the difference in allele frequencies was statistically significant (P < 0.001), the recessive allele for skin colour (w) was considerably more frequent than the dominant W allele (0.60 vs. 0.40) (Table). Since this trait follows a pattern of complete dominance, approximately 64% of the birds exhibited white skin, while 36% displayed yellow skin. This result concurs with earlier reports from India (Agarwal *et al*., 2020) and other tropical countries (Tabassum *et al*., 2014; Melesse & Negesse, 2011; Bibi *et al*., 2021). Recent studies demonstrated that the dominant *W* allele suppresses carotenoid accumulation by upregulating *BCO2* gene expression, resulting in white skin. In contrast, the recessive *w* allele permits carotenoid deposition in the skin, producing a yellow phenotype (Eriksson *et al*., 2008). The presence of both white and yellow skin in the indigenous chickens of India is believed to have originated from ancestral hybridization between the white-skinned red junglefowl and the yellow-skinned grey junglefowl (Eriksson *et al*., 2008).

**3.4 Feather structure**

Frizzling is a structural variation in chicken feathers characterized by a pronounced curvature in the rachis, or central shaft, causing the feathers to curl outward and upward rather than lying flat against the body (Carter and Matheson, 1962). Frizzle phenotype has been associated with improved thermoregulation due to better air contact with skin, enabling better performance and viability under hot and humid climatic conditions (Gowe and Fairfull, 1995). In the present study, however, the frequency of frizzled birds was remarkably low (1%), with an extremely low allelic frequency of the frizzle gene (*F*) at 0.005 (Table). Similar low frequencies of frizzled birds among indigenous chicken populations have been reported in different parts of the world (Fajemilehin, 2010; Hassaballah *et al*., 2014; Dahloum *et al*., 2016). Despite the known adaptive advantage of the frizzle gene under heat stress, its low prevalence in tropical region like India may be due to socio-cultural factors. In some communities, frizzle-feathered birds are perceived as less attractive and command lower market value (Yakubu, 2010). Additionally, certain traditional beliefs associate these birds with witchcraft or rituals, further discouraging their rearing (Fajemilehin, 2010). Genetically, the low frequency may also be attributed to the semi-lethal nature of the frizzle gene in the homozygous state (*FF*), which can result in reduced viability (Haaren-Kiso *et al*., 1995). Molecular studies have now revealed that the frizzle phenotype arises from a mutation in the *KRT75* gene, which encodes α-keratin, a major structural protein in feathers, leading to the characteristic twisting and bending of the rachis (Ng *et al*., 2012).

**3.5 Feather distribution**

 Naked neck and ptilopody (feathered shank) are two commonly observed traits in chickens that involve either the absence of feathers from a typical body region or the presence of feathers in an atypical location.

The naked neck trait is governed by an incompletely dominant gene, in which birds with the homozygous dominant genotype (*NaNa*) exhibit a completely bare neck, heterozygotes (*Nana*) show partial feathering and those with the homozygous recessive genotype (*nana*) have a fully feathered neck (Somes, 1969). In the present study, only 1% of birds displayed partial naked neck phenotype, resulting in an estimated allele frequency of 0.005 for *Na* and 0.995 for *na* (Table). Similarly, low frequencies of the *Na* allele have been reported in indigenous chicken populations from various regions; 0.045 in Algeria (Dahloum *et al*., 2016), 0.05 in Ghana (Mensah *et al*., 2023) and between 0.037 and 0.051 in Nigeria (Fayeye *et al*., 2006). A relatively higher frequency of 0.197 has been documented in Indonesian native chickens (Setianto *et al*., 2009). The adaptive advantage of the naked neck gene in tropical climates is well documented, particularly in improving thermoregulation and productivity under heat stress conditions (Gowe and Fairfull, 1995). Despite its clear physiological benefits, the prevalence of this trait remains low in most indigenous populations. This discrepancy is likely due to human-driven negative selection, as the appearance of exposed skin is often perceived as unaesthetic or undesirable. As a result, aesthetic preferences override natural selection, contributing to a significant (P < 0.001) net imbalance between allelic frequencies.

Ptilopody in chickens refers to the presence of feathers on the shanks and toes, a condition also known as feathered shanks. This trait is governed by an incompletely dominant gene (*Pti*), where the homozygous dominant genotype results in full feathering, the heterozygous condition leads to partial feathering, and the homozygous recessive genotype produces clean, non-feathered shanks (Somes, 1990). In the present study, only 1% of the indigenous chickens of Kerala exhibited heterozygous ptilopody, indicating a very low gene frequency for the dominant allele (*Pti*) at 0.008, and a high frequency for the recessive allele (*pti*) at 0.992 (Table 1). This significant (P < 0.001) imbalance between dominant and recessive alleles aligns with reports from other countries, including Ghana (0.01 Vs. 0.99 %; Mensah *et al*., 2023), Algeria (0.006 Vs. 0.994%; Dahloum *et al*., 2016), and Nigeria (0.076 Vs. 0.924 %; Sola-Ojo *et al*., 2011). In contrast, Larivière and Leroy (2010) observed a much higher prevalence of ptilopody (19.4%) in Belgian indigenous chickens, suggesting regional variation in both the frequency and acceptance of this trait. The markedly low frequencies in most tropical regions may be attributed to socio-cultural preferences, where feathered shanks are often viewed as undesirable, leading to negative selection and gradual elimination of the trait from indigenous chicken populations.

**3.6 Feather length**

Crest is a phenotypic trait in chickens, characterized by a prominent tuft of feathers on the top of the head, giving the bird a distinctive “crowned” appearance. This trait is governed by an autosomal incompletely dominant gene (*Cr*). Chickens with the homozygous dominant genotype (*CrCr*) exhibit a well-developed crest, while heterozygous individuals (*Crcr*) display a moderate crest. Birds with the homozygous recessive genotype (*crcr*) lack the crest and have normal head feathering (Somes, 1990). In the present study, only 2% of birds were heterozygous for the crest gene, resulting in a very low frequency of the dominant allele (*Cr* = 0.01) and a high frequency of the recessive allele (*cr* = 0.99) (Table 1). Similar observations of low prevalence of the crest gene in indigenous chicken populations have been reported in tropical countries namely, 0.074 in Nigeria (Sola-Ojo *et al*., 2011) and 0.03 in Algeria (Dahloum *et al*., 2016). In contrast, a relatively high frequency of crested birds (20.1%) was observed among indigenous chickens in Belgium (Larivière and Leroy, 2010). This disparity suggests a strong negative selection pressure against the crest trait in tropical regions, likely due to farmer preference or perceived disadvantages, leading to a significant (P < 0.001) imbalance in allele frequencies between the dominant and recessive forms.

**4. CONCLUSION**

It can be concluded that the indigenous chickens of Kerala exhibit remarkable phenotypic diversity, particularly in traits influenced by the *E* locus and associated plumage patterns. However, the prevalence of other qualitative traits controlled by major genes—such as frizzling, naked neck, ptilopody, and crest was notably low, with significant deviations from presumed equal frequency of dominant and recessive alleles under Hardy–Weinberg equilibrium. This suggests an underlying trend of negative selection, possibly driven by socio-cultural preferences and market-driven biases against certain visible traits. Despite their known adaptive benefits in tropical environments, traits like naked neck and frizzling remain underrepresented, raising concerns about genetic erosion. The data affirm the value of these native chickens as reservoirs of rare and functionally important alleles. Immediate attention is required to document, conserve, and utilize these genetic traits to ensure their availability for future breeding programs, particularly in the face of climate change and evolving production demands.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

The authors used ChatGPT (OpenAI, 2025) for language editing and improvement of clarity. The authors confirm that the content and scientific interpretations are their own.

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**Table 1: Prevalence of genotypes of major genes and their alleles in indigenous chicken of Kerala**

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| --- | --- | --- | --- |
| **Character** | **Variants** | **Genotypes (n=200)** | **Alleles (n=400)** |
| **Genotypes** | **Obser-ved** | **Expe-cted** | **Chi square** | **Per-centage** | **Alleles** | **Calculated allelic frequency** | **Calculated no. of alleles** | **Expected no. of alleles** | **Chi square** |
| Comb type  | Pea (equal height ridges) | *PP* | 0 | 50 | 560.67\*\*\*  | 2.50 | P | 0.013 | 10.33 | 200.00 | 359.86\*\*\*  |
| Pea (higher middle ridge) | *Pp* | 5 | 100 | 97.50 | p+ | 0.987 | 389.67 | 200.00 |
| Single  | *pp* | 195 | 50 |  |  |  |  |  |  |
| **Total** |  | **200** | **200** |  | **100.00** |  | **1.000** | **400.00** | **400.00** |  |
| Skin colour | White | *WW & Ww* | 128 | 150 | 12.91\*\*\*  | 64.00 | W+ | 0.400 | 256.00 | 200.00 | 31.36\*\*\*  |
| Yellow | *ww* | 72 | 50 | 36.00 | w | 0.600 | 144.00 | 200.00 |
| **Total** |  | **200** | **200** |  | **100.00** |  | **1.000** | **400.00** | **400.00** |  |
| Feather structure | Extreme frizzling (lethal) | *FF* | 0 | 0 | 385.16\*\*\*  | 0.00 | F | 0.005 | 3.99 | 132.00 | 185.28\*\*\*  |
| Moderate frizzling  | *Ff* | 2 | 133 | 1.00 | f+ | 0.995 | 396.01 | 268.00 |
| Normal | *ff* | 198 | 67 | 99.00 |   |   |   |   |   |
| **Total** |  | **200** | **200** |  | **100.00** |  | **1.000** | **400.00** | **400.00** |  |
| Feather distribution | Complete naked neck  | *NaNa* | 0 | 50 | 584.12\*\*\*   | 0.00 | Na | 0.005 | 3.99 | 200.00 | 185.28\*\*\*  |
| Partial naked neck | *Nana* | 2 | 100 | 1.00 | na+ | 0.995 | 396.01 | 200.00 |
| Normal | *nana* | 198 | 50 | 99.00 |   |   |   |   |   |
| **Total** |  | **200** | **200** |  | **100.00** |  | **1.000** | **400.00** | **400.00** |  |
| Feather distribution | Complete ptilopody | *PtiPti*  | 0 | 50 | 576.27\*\*\*  | 0.00 | Pti | 0.008 | 6.37 | 200.00 | 374.92\*\*\*  |
| Partial ptilopody | *Ptipti* | 3 | 100 | 1.50 | pti+ | 0.992 | 393.63 | 200.00 |
| Clean shank | *ptipti* | 197 | 50 | 98.50 |  |  |  |  |
| **Total** |  | **200** | **200** |  | **100.00** |  | **1.000** | **400.00** | **400.00** |  |
| Feather length | Large crest | *CrCr*  | 0 | 50 | 568.48\*\*\*  | 0.00 | Cr | 0.010 | 7.96 | 200.00 | 368.79\*\*\*  |
| Moderate crest | *CRcr* | 4 | 100 | 2.00 | cr+ | 0.990 | 392.04 | 200.00 |
| Normal head | *crcr* | 196 | 50 | 98.00 |  |  |  |  |
| **Total** |  | **200** | **200** |  | **100.00** |  | **1.000** | **400.00** | **400.00** |  |
| Feather pattern | Columbian restriction | *CoCo & Coco* | 3 | 150 | 576.12\*\*\* | 1.50 | Co | 0.008 | 6.37 | 200.00 | 374.92\*\*\*  |
| Normal | *coco* | 197 | 50 |  | 98.50 | co+ | 0.992 | 393.63 | 200.00 |  |
| **Total** |  | **200** | **200** |  | **100.00** |  | **1.000** | **400.00** | **400.00** |  |

 \*\*\* Significant (P<0.001); + - Wild allele