

Phenotypic Analysis of Growth and Morphological Traits in Miniature Breeds of Japanese Indigenous Chickens

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Japanese indigenous chickens include approximately 50 breeds exhibiting various morphological traits, such as a long tail. These genetic resources will be important for revealing the genetic basis of morphological traits in the future. However, little is known about the phenotypic characteristics of each breed during the growth stages. To understand age-dependent changes in growth and morphological traits, we investigated tail length, tail number, body weight, and shank length at several time points using three genetically distinct Japanese indigenous chicken breeds. A total of 155 birds from the Tosa-jidori, Chabo, and Minohikichabo breeds were used for trait measurements from 1 to 36 weeks of age to reveal breed and sex effects. Significant sex differences through the growth stages were observed for all traits except for tail number. Although there were no clear breed differences in tail length traits at the 6- and 20-week stages, Minohikichabo ultimately had a significantly longer tail due to extended tail feather growth at later stages (28 and 36 weeks). By measuring two tail length variables (central and maximum), it was revealed that the shape of the tail feathers varies with the growth stage. Minohikichabo's tail number was higher than that of Tosa-jidori and Chabo at earlier ages (8 and 16 weeks), which leads to an elegant visual in Minohikichabo. Tosa-jidori's body weight was higher than that of Chabo and Minohikichabo, whereas the shank lengths of Chabo and Minohikichabo were shorter than those of Tosa-jidori. These differences in body weight and shank length were consistent from the early to late growth stages. These results revealed the age-dependency of growth and morphological trait breed characteristics.

Key words: age-dependent change, chicken, Japanese indigenous breed, tail, growth, phenotyping

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Introduction

Red jungle fowl (*Gallus gallus*), the main wild ancestor of chickens, inhabits India and Southeast Asia (Liu *et al.*, 2006; Peters *et al.*, 2016). Approximately 9,500 years ago, the red jungle fowl subspecies *Gallus gallus spadiceus* has been domesticated by humans (Wang *et al.*, 2020), and consequently, chickens have spread throughout the world. Sub-

sequently, chickens were selected for several purposes, including utility, i.e., meat and egg production, and ornamental purposes, i.e., crowing observation and feathering features (Ekariu, 2007). Economically important growth, meat, and egg traits, which are quantitative traits, are controlled by several quantitative trait loci (QTLs) and environmental factors (Mackay, 2014). If QTLs for economic traits are revealed, information on marker genotypes near the QTLs can be applied to selectively breed for those traits (Lande and Thompson, 1990; van der Beek and van Arendonk, 1996). Therefore, it is important to understand the genetic basis of growth traits in the livestock industry.

Japanese indigenous chickens are classified into two groups: fancy chickens for hobbyists and utility chickens for egg and meat production. Although there are approximately 50 indigenous chicken breeds in Japan, these breeds are primarily categorized as fancy chickens (Tsudzuki, 2003). Japanese fancy chickens reared by hobbyists to appreciate

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the morphologies of their bodies and plumage, behaviors, and prolonged crowing (Tsudzuki, 2003), have different genetic roots from the European and American egg- and meat-type breeds in the poultry industry (Osman *et al.*, 2006). Japanese fancy chickens have various distinct morphological characteristics, particularly tail feathers. In the present study, we focused on three Japanese chicken breeds, Japanese Old Type-Tosa (Tosa-jidori), Japanese Bantam (Chabo), and Japanese Tail Dragger (Minohikichabo), to analyze tail feather traits—all three breeds are classified as small breeds. Tosa-jidori, one of the oldest breeds, has a body shape and plumage color similar to those of the wild ancestor; the tail feather morphology exhibits a curve, which is categorized as wild-type (Fig. 1a and 1b). Chabo has erect-type tail feathers and short shanks (Figs. 1c and 1d). Minohikichabo roosters have considerably long tail feathers (Tsudzuki, 2003), a tail feather morphology categorized as rich-type, exhibiting a large number of long tails by visual observation (Figs. 1e and 1f).

As stated above, the unique morphology of these breeds in adult chickens has been described. However, detailed features of growth and tail feather traits have not been analyzed at different time points from chicks to adult chickens in either sex. Since body weight and shank length are representative indicators of body size, simultaneous phenotyping of both tail and body size traits will be crucial for further genetic analyses. In our preliminary studies, there was no clear difference in adult tail length between a breed (3–4 kg body weight) and Minohikichabo (less than 1 kg body weight), indicating that body size traits are crucial for comparisons of

tail phenotype genetic factors. Given that the genetic basis of body size and tail feathers in Japanese fancy chickens are fully understood, morphological traits could be precisely altered through breeding using the available genetic resources, leading to substantial benefits for the poultry industry and fancier breeders. The quantification of traits and detailed phenotypic analyses are needed to reveal the genetic basis of these traits (Goto and Tsudzuki, 2017).

In the present study, we aimed to investigate the growth patterns of morphological traits at several time points using three genetically distinct chicken breeds. This will be a substantial step in revealing breed and sex differences in morphological traits, especially tail feather traits. Moreover, this fundamental data will be applied for future genomics studies to reveal the genes underlying morphological traits in chickens.

Materials and Methods

Animals

A total of 155 birds from the Tosa-jidori (male, $n=35$; female, $n=37$), Chabo (male, $n=11$; female, $n=12$), and Minohikichabo (male, $n=21$; female, $n=39$) breeds were investigated. The chickens were reared with free access to feed and water in individual cages and a photoperiod cycle of 16 hr light and 8 hr dark. Management was performed according to the rules of Standards Related to the Care and Management of Experimental Animals (Prime Ministers' Office, Japan, 1980) and the Guide for the Use of Experimental Animals in Universities (The Ministry of Education, Science, Sports, and Culture, Japan, 1987). This study (authorization number 20-139) was approved by the Experi-

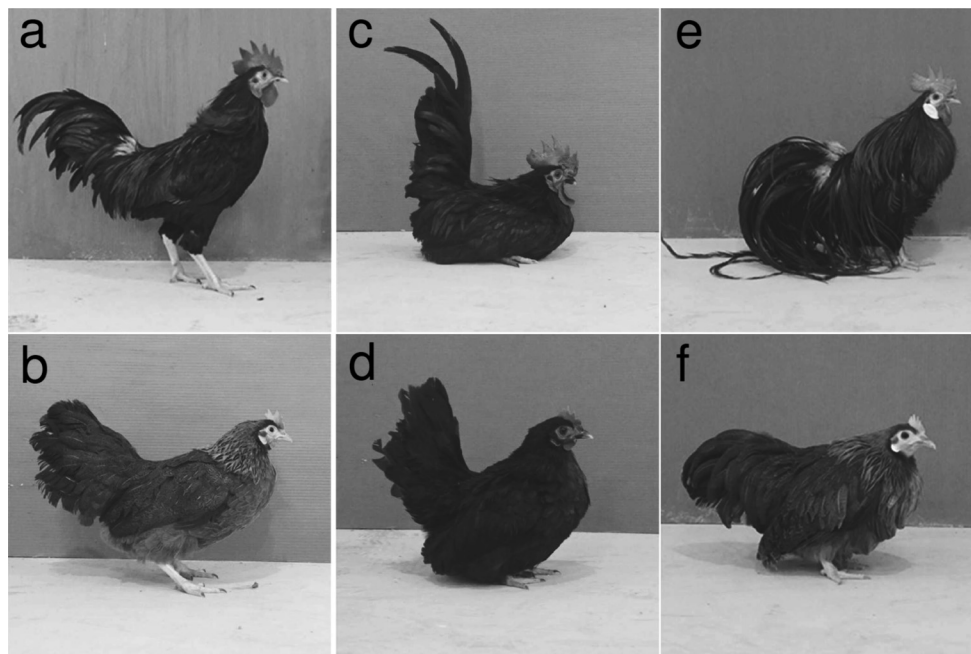


Fig. 1. **Japanese fancy chicken breeds used in this study.** (a) Male Tosa-jidori, (b) Female Tosa-jidori, (c) Male Chabo, (d) Female Chabo, (e) Male Minohikichabo, (f) Female Minohikichabo.

mental Animal Committee of the Obihiro University of Agriculture and Veterinary Medicine.

Phenotypic Measurement

The target phenotypes were body weight (BW), tail length (TL), shank length (SL), and tail number (TN). Tail length was measured using a ruler. The main bird feathers consist of contour feathers (composed of feather branches, rachises, and vanes) and down. We defined the tail length of natal down (observed in newborn chicks) as 0 mm. We measured the contour feather length (from the feather branch to the vane's tip). To evaluate the morphological variation of tail feathers, we measured both central tail length and maximum tail length. BW was measured using an electronic balance (WPB3KO1; AS ONE Co., Osaka, Japan). Shank length (from the joint of the tarsal bone to the metatarsal bone) was measured using a digital caliper (1-894-02; AS ONE Co.). Tail numbers were counted by manual observation of tail feathers located in the area from the uropygial gland to the cloaca. Central tail length (C_TL), maximum tail length (M_TL), BW, and SL were collected weekly from 1 to 20 weeks of age (wk) to estimate growth at several stages. After measuring for 20 weeks, trait measurements were collected every 4 weeks up to 36 weeks. The traits of interest were C_TL, M_TL, BW, and SL at 6, 20, 28, and 36 weeks to examine the main effects of breed and sex and their interaction effects. TN was determined at 8 and 16 weeks.

Statistics

Statistical analyses were conducted in two steps from the data of each trait in the Tosa-jidori, Chabo, and Minohikichabo breeds. As a first step, we analyzed each trait using C_TL, M_TL, BW, and SL by two-way analysis of variance (ANOVA) to test the main and interaction effects of breed and sex at each stage (6, 20, 28, and 36 weeks). TN at 8 and 16 weeks was also analyzed by two-way ANOVA to determine the breed and sex effects. If there was a significant difference ($P < 0.05$), a post-hoc test was performed using Tukey's HSD test.

After 6 weeks, a significant sex effect (sexual dimorphism) was observed using two-way ANOVA. Therefore, we conducted a second analysis separately for each sex. Data for each sex were analyzed by two-way mixed-design ANOVA with breed (Tosa-jidori, Chabo, and Minohikichabo) as the between-subjects factor and age (several weeks of age) as the within-subject (repeated) factor (Mori *et al.*, 2020; Goto *et al.*, 2019a) to determine the main effects of breed, age, and their interaction effect. If there was a significant difference ($P < 0.05$), Tukey's HSD test was performed.

In addition, Pearson's correlation coefficients among traits were calculated and expressed using the *corrplot* (Wei and Simko, 2017) and *ggplot2* (Wickham, 2016) packages of R (R Core Team, 2019) using a threshold of $P < 0.05$. Statistical analyses were performed using R Studio (ver. 3. 6. 1).

Results

Effects on Breed and Sex in Growth and Morphological Traits at Several Stages

The main and interaction effects of breed and sex were

tested in the central tail length (C_TL) trait (Fig. 2a-2d and Table S1). At 6 weeks, there were significant sex effects ($F_{1,149} = 13.656$, $P = 0.000308$), but no breed effects ($F_{2,149} = 0.412$, $P = 0.66$) or interactions ($F_{2,149} = 0.209$, $P = 0.81$). There was also a significant sex effect on C_TL at 20 weeks ($F_{1,114} = 96.174$, $P < 2e-16$), but no breed ($F_{2,114} = 0.113$, $P = 0.89$) or interaction effects ($F_{2,114} = 2.688$, $P = 0.07$). In contrast, the two-way ANOVA for C_TL revealed significant breed ($F_{2,78} = 6.703$, $P = 0.002060$) and sex effects ($F_{1,78} = 207.609$, $P < 2e-16$), and their interaction ($F_{2,78} = 8.084$, $P = 0.000645$) at 28 weeks. At 36 weeks, there were also significant breed ($F_{2,71} = 14.88$, $P = 4.01e-06$) and sex effects ($F_{1,71} = 275.68$, $P < 2e-16$), and interaction effects ($F_{2,71} = 30.75$, $P = 2.40e-10$). Although there was no difference among breeds at 6 and 20 weeks, Minohikichabo had longer C_TL than the others at 28 and 36 weeks. Males had a longer C_TL than females at all stages. Significant breed and sex interaction effects were found at 28 and 36 weeks, with Minohikichabo roosters exhibiting remarkably longer tail lengths.

The main and interaction effects of breed and sex were tested for the maximum tail length (M_TL) trait (Fig. 2e-2h and Table S2). At 6 weeks, there were significant breed ($F_{2,149} = 13.051$, $P = 5.99e-06$) and sex effects ($F_{1,149} = 10.903$, $P = 0.0012$), but no interaction ($F_{2,149} = 0.937$, $P = 0.39$). In M_TL at 20 weeks, a significant sex effect ($F_{1,114} = 135.587$, $P < 2e-16$) was identified, but no difference was found on breeds ($F_{2,114} = 0.077$, $P = 0.93$) and interaction effect ($F_{2,114} = 1.191$, $P = 0.31$). In M_TL at 28 weeks, there were significant breed ($F_{2,83} = 6.427$, $P = 0.002541$), sex ($F_{1,83} = 252.333$, $P < 2e-16$), and interaction effects ($F_{2,83} = 10.273$, $P = 0.000103$). At 36 weeks, M_TL was significantly affected by breed ($F_{2,71} = 15.78$, $P = 2.31e-06$), sex ($F_{1,71} = 292.03$, $P < 2e-16$), and their interaction ($F_{2,71} = 35.98$, $P = 1.62e-11$). Chabo had longer M_TL than Minohikichabo and Tosa-jidori at 6 weeks, whereas there was no difference among breeds at 20 weeks. At 28 and 36 weeks, Minohikichabo had longer M_TL than Tosa-jidori and Chabo. Males had a longer M_TL than females at all stages. Minohikichabo males had remarkably longer tails at later stages.

For the body weight (BW) trait, the main and interaction effects of breed and sex were tested (Fig. 2i-2l and Table S3). At 6 weeks, significant breed ($F_{2,148} = 41.026$, $P = 6.67e-15$) and sex effects ($F_{1,148} = 30.863$, $P = 1.25e-07$), but no interaction ($F_{2,148} = 1.385$, $P = 0.25$) were detected. At 20 weeks of age, there were significant breed ($F_{2,114} = 53.20$, $P < 2e-16$), sex ($F_{1,114} = 90.72$, $P = 3.56e-16$), and interaction effects ($F_{2,114} = 10.14$, $P = 8.84e-05$). At 28 weeks of age, the two-way ANOVA revealed significant breed ($F_{2,83} = 65.87$, $P < 2e-16$), sex ($F_{1,83} = 89.08$, $P = 8.67e-15$), and interaction effects ($F_{2,83} = 15.62$, $P = 1.74e-06$). At 36 weeks of age, significant breed ($F_{2,71} = 51.25$, $P = 1.68e-14$), sex ($F_{1,71} = 64.77$, $P = 1.36e-11$), and interaction effects ($F_{2,71} = 11.19$, $P = 5.98e-05$) were found. Tosa-jidori was heavier than Minohikichabo at all stages, while males were heavier than females at all stages. Sexual differences in Tosa-jidori were higher than in the other breeds at 20, 28, and 36 weeks,

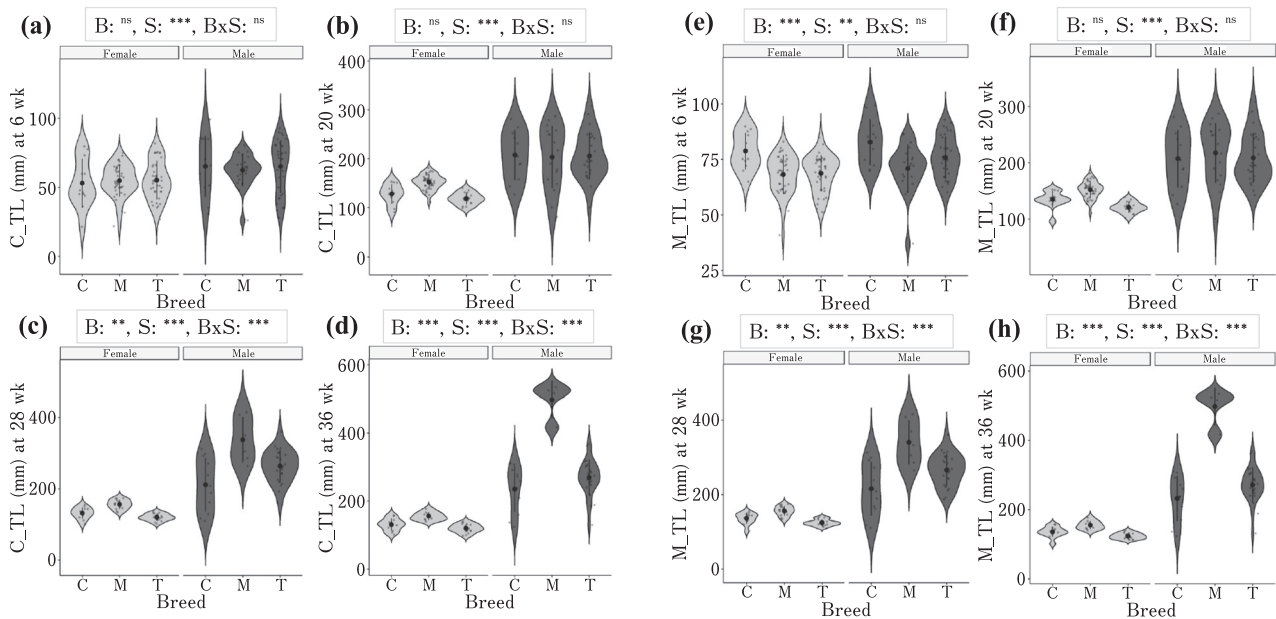


Fig. 2. Effects on breed and sex of growth and morphological traits in Tosa-jidori, Chabo, and Minohikichabo. Tosa-jidori is denoted by T (male $n=35$, female $n=37$), Chabo denoted by C (male $n=11$, female $n=12$), Minohikichabo denoted by M (male, $n=21$, female $n=39$). A violin plot was used to show the distribution of quantitative data. Larger black dots and bars indicate mean \pm SD for each breed and sex, whereas smaller dots indicate individual values. Statistical results are shown as the main effects of breed (B), sex (S), and interaction effect (B \times S). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, and ^{ns} $P > 0.05$. (a–d) For central tail length (C_TL), two-way ANOVA revealed significant breed main effects at 28 and 36 weeks, sex main effects at all stages, and a breed-sex interaction effect at 28 and 36 weeks. (e–h) For maximum tail length (M_TL), two-way ANOVA indicated breed effects at 6, 28, and 36 weeks, sex effects at all stages, and their interaction effect at 28 and 36 weeks. (i–l) For body weight (BW), two-way ANOVA revealed significant breed and sex effects at all stages and interaction effects at 20, 28, and 36 weeks. (m–p) For shank length (SL), two-way ANOVA indicated significant breed and sex effects at all stages. (q, r) For tail number (TN), significant breed effects at 8 and 16 weeks were found.

whereas those in Chabo was relatively small.

For the shank length (SL) trait, the main and interaction effects of breed and sex were tested (Fig. 2m–2p and Table S4). Significant main effects were detected at 6 weeks (breed; $F_{2,130}=75.521$, $P < 2e-16$, sex; $F_{1,130}=17.520$, $P=5.2e-05$), 20 weeks (breed; $F_{2,95}=64.994$, $P < 2e-16$, sex; $F_{1,95}=55.972$, $P=3.67e-11$), 28 weeks (breed; $F_{2,64}=54.979$, $P=1.27e-14$, sex; $F_{1,64}=31.824$, $P=4.12e-07$), and 36 weeks (breed; $F_{2,52}=46.527$, $P=2.61e-12$, sex; $F_{1,52}=26.688$, $P=3.84e-06$). There were no interaction effects at 6 weeks ($F_{2,130}=0.491$, $P=0.61$), 20 weeks ($F_{2,95}=2.194$, $P=0.12$), 28 weeks ($F_{2,64}=2.894$, $P=0.06$), and 36 weeks ($F_{2,52}=2.654$, $P=0.08$). Tosa-jidori had longer SL than Chabo and Minohikichabo, whereas males had longer SL than females at all stages.

The main and interaction effects of breed and sex on the tail number (TN) trait were determined (Fig. 2q, 2r and Table S5). At 8 weeks, a significant breed effect ($F_{2,150}=127.845$, $P < 2e-16$) was observed, but no sex ($F_{1,150}=0.859$, $P=0.36$) and interaction effects ($F_{2,150}=1.050$, $P=0.35$)

were observed. Breed had a significant effect on TN at 16 weeks ($F_{2,119}=108.682$, $P < 2e-16$), whereas neither sex ($F_{1,119}=0.647$, $P=0.42$) nor interaction effects ($F_{2,119}=0.014$, $P=0.99$) were observed. Minohikichabo had a larger tail number than Tosa-jidori and Chabo at both stages.

Age-dependent Changes in Growth and Morphological Traits

Age-dependent changes in C_TL were evaluated from 1 to 36 weeks (Fig. 3a). Main and interaction effects of breed and age were tested using two-way ANOVA for each sex. In males, there were significant breed ($F_{2,55}=4.633$, $P=0.0138$), age ($F_{23,1283}=375.24$, $P < 2e-16$), and interaction effects ($F_{46,1283}=10.49$, $P < 2e-16$). In females, significant breed ($F_{2,78}=34.28$, $P=2.08e-11$), age ($F_{23,1624}=774.33$, $P < 2e-16$), and interaction effects ($F_{46,1624}=18.04$, $P < 2e-16$) were observed. C_TL extended as growth progressed. Minohikichabo had a longer tail than the other breeds, and the differences were clearly seen from 24–28 weeks.

To determine age-dependent changes in M_TL from 1–36 weeks, two-way ANOVA for each sex was used to evaluate

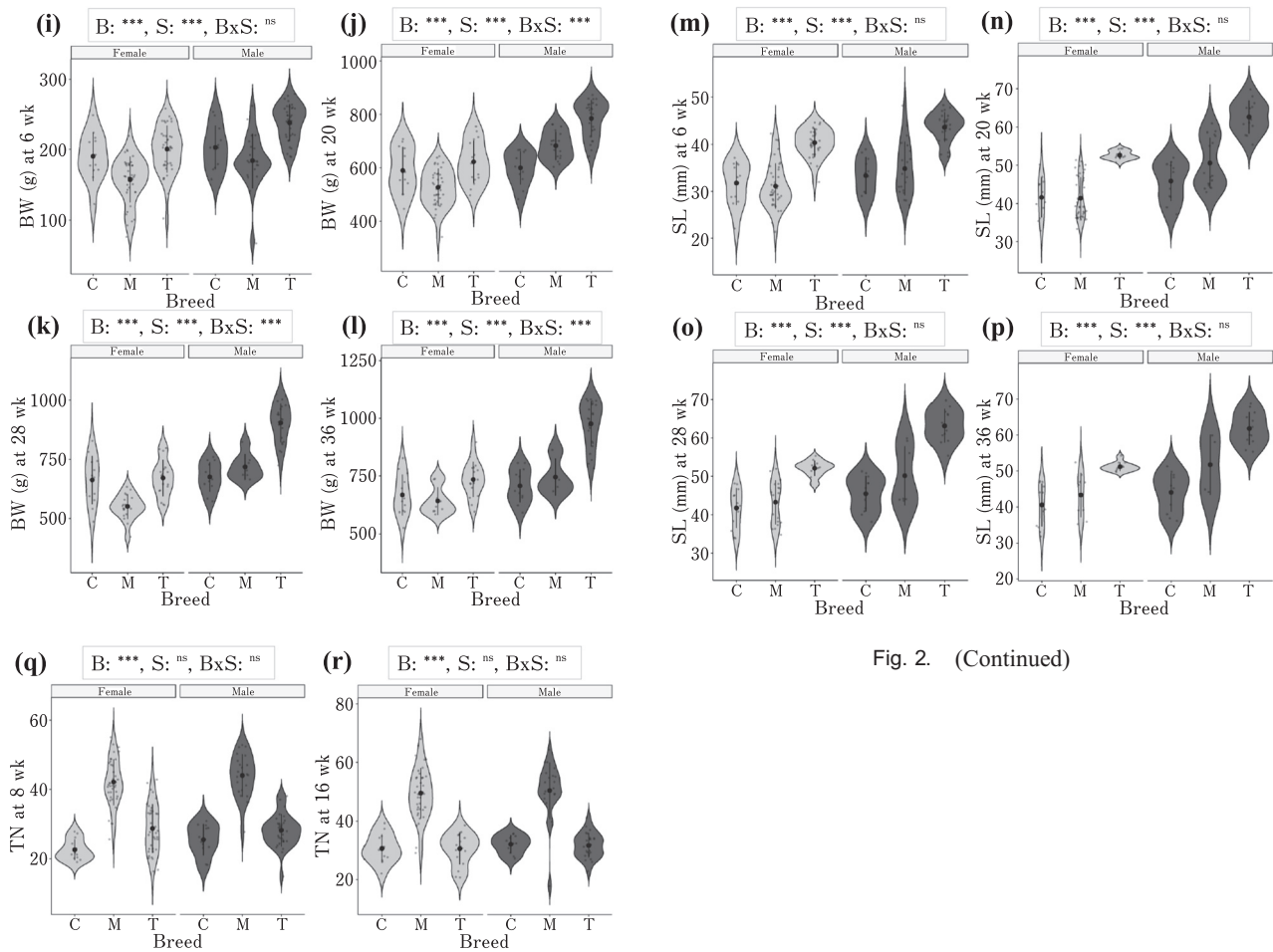


Fig. 2. (Continued)

the main and interaction effects of breed and age (Fig. 3b). In males, the two-way ANOVA revealed significant breed ($F_{2,54}=4.075$, $P=0.0225$), age ($F_{23,1259}=449.20$, $P<2e-16$), and interaction effects ($F_{46,1259}=13.68$, $P<2e-16$). In females, significant breed ($F_{2,77}=34.53$, $P=1.98e-11$), age ($F_{23,1614}=1355.54$, $P<2e-16$), and interaction effects ($F_{46,1614}=24.67$, $P<2e-16$) were detected. M_TL extended as the growth progressed. Minohikichabo had a longer tail and C_TL than the other breeds at later stages.

Age-dependent changes in BW were evaluated from 1–36 weeks (Fig. 3c). The main and interaction effects of breed and age were tested using two-way ANOVA for each sex. In males, significant breed ($F_{2,55}=33.660$, $P=2.84\cdot 10^{-10}$), age ($F_{23,1283}=3412.96$, $P<2e-16$), and interaction effects ($F_{46,1283}=26.94$, $P<2e-16$) were identified. In females, there were significant breed ($F_{2,78}=16.61$, $P=9.76e-07$), age ($F_{23,1628}=2403.62$, $P<2e-16$), and interaction effects ($F_{46,1628}=12.75$, $P<2e-16$). BW increased with growth. Tosa-jidori males were heavier than the other breeds, whereas Minohikichabo females were lighter than the other breeds.

We evaluated age-dependent changes in SL from 1–36 weeks (Fig. 3d). Two-way ANOVA for each sex determined the main and interaction effects of breed and age. Significant breed ($F_{2,41}=37.217$, $P=6.09e-10$), age ($F_{23,988}$

$=978.5$, $P<2e-16$), and interaction effects ($F_{46,988}=15.0$, $P<2e-16$) were found in males, while significant breed ($F_{2,68}=29.131$, $P=7.28e-10$), age ($F_{23,1438}=1151.41$, $P<2e-16$), and interaction effects ($F_{46,1438}=13.68$, $P<2e-16$) were observed in females. SL increased with growth, and shank length seemed to reach a plateau around 13 weeks in each breed and sex. Tosa-jidori had a longer shank than the others in both males and females.

Phenotypic Correlation Analysis

Phenotypic correlations were estimated using several ages of the C_TL (Fig. S1 and S2). There were positive correlations among C_TL traits measured within 2–3 weeks (such as 7 and 8 wk and 19 and 20 weeks). On the other hand, negative phenotypic correlations were seen among traits, which had a 5–10-week age difference (e.g., 4 and 9 weeks, 4 and 13 weeks, and 5 and 12 weeks). In addition, traits at 1 and 2 weeks were positively correlated with traits at 10–15 weeks, whereas there were negative correlations between traits at 1 and 2 weeks and 28–36 weeks. Similar tendencies were observed for the phenotypic correlations of M_TL (Fig. S3 and S4). Tail length at early stages could not be used to estimate the length at the adult stages.

For BW (Fig. S5 and S6), phenotypic correlations between traits across ages were estimated for each sex. There were

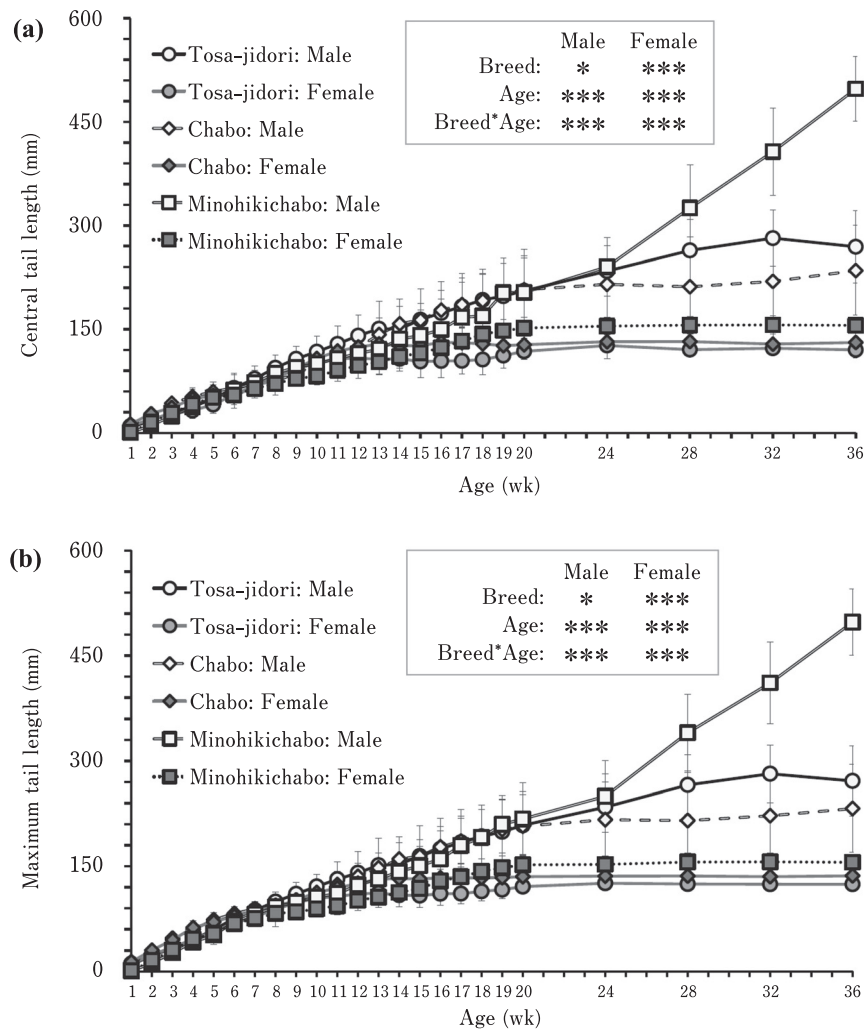


Fig. 3. Age-dependent changes in growth and morphological traits in Tosa-jidori, Chabo, and Minohikichabo. Plots and bars indicate the mean \pm SD for each breed and sex. Tosa-jidori (male, $n=35$; female, $n=37$), Chabo (male, $n=11$; female, $n=12$), and Minohikichabo (male, $n=21$; female, $n=39$) were used. * $P<0.05$ and *** $P<0.001$. (a) Central tail length (C_TL) for each breed and sex (b) Maximum tail length (M_TL) for each breed and sex. (c) Body weight (BW) of each breed and sex. (d) Shank length (SL) of each breed and sex. Two-way ANOVA revealed significant breed and age main effects, and interaction effects in all traits in both males and females.

broadly positive correlations among traits at several stages. A similar tendency was observed for SL (Fig. S7 and S8); traits between 7–36 weeks were highly positively correlated, indicating that shank length at the adult stage can be estimated from around 7 weeks.

We checked the phenotypic correlations among the traits as shown in Figs. S9 and S10. In males, BW and SL were positively correlated, regardless of the growth stage. C_TL and M_TL were positively correlated at the same age. Longer tail lengths at later stages (28 and 36 weeks) tended to have a higher number of tails. These tendencies were also observed in females. No or weak negative correlations were detected between body size traits (BW and SL) and tail traits

(C_TL, M_TL, and TN) in males and females, respectively.

Discussion

In the present study, growth and morphological traits (tail length, tail number, body weight, and shank length) were investigated at several time points using three genetically distinct indigenous Japanese chicken breeds. Significant sex differences through the growth stages were observed for all traits except for tail number. Although there were no clear breed differences in tail length traits at the 6- and 20-week stages, Minohikichabo ultimately had significantly longer tails via extended growth of tail feathers at later stages. By measuring two parts of tail length (central; C_TL and

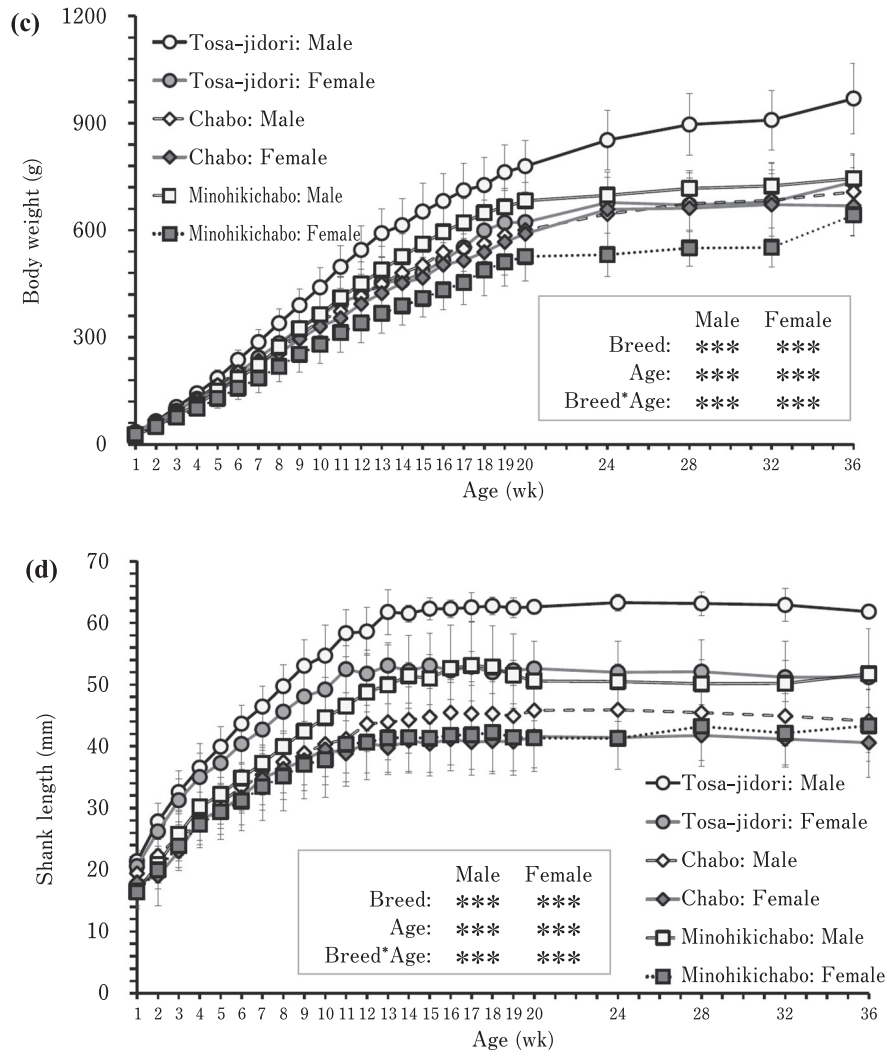


Fig. 3. (Continued)

maximum; M_{TL}), it was revealed that the shape of tail feathers varies with the growth stage. Minohikichabo's tail number was higher than that of Tosa-jidori and Chabo at 8 and 16 weeks. The body weights of Tosa-jidori were higher than those of Chabo and Minohikichabo, while the shank lengths of Chabo and Minohikichabo were shorter than those of Tosa-jidori. These differences in body weight and shank length were consistent between the early and late growth stages. These results clearly show breed characteristics in age-dependent changes in growth and morphological traits.

This study revealed that Minohikichabo has a larger number of tails than the other breeds from earlier ages, which will lead to an elegant visual in Minohikichabo (Tsudzuki, 2003). In general, the number of natal downs in the tail feathers of chickens increases with time. Subsequently, the tail feathers are pushed by contour feathers from the feather follicle. Throughout the growth stages, we observed two types of tail feather shapes, fan- (Fig. 4a) and crescent-shaped tail feathers (Fig. 4b). Fan-shaped tail feathers in-

dicate that the maximum tail feather equals the central tail, which means that the maximum tail feather is observed on the central tail. On the other hand, crescent-shaped tail feathers indicate that the maximum tail feather is longer than the central tail, which means that the maximum tail feather is observed at the left- and right-side tails. It has been revealed that tail feathers gradually molt from the center to the sides in birds with crescent-shaped tail feathers. Since natal downs in central tails will be molted earlier than those in both side tails, crescent-shaped tail feathers could be observed in the current study. After the late stage, fan-shaped tail feathers (maximum tail feathers equal to the central tail) were observed in both sexes in all three breeds, but sexual differences could be found by visual observation. Females in all breeds showed typical fan-shaped tail feathers, whereas males in all breeds exhibited fan-shaped tail feathers with relatively longer central tails. In particular, Minohikichabo males have remarkably longer central tails. In the present study, detailed phenotypic analyses of tail length at several time points could

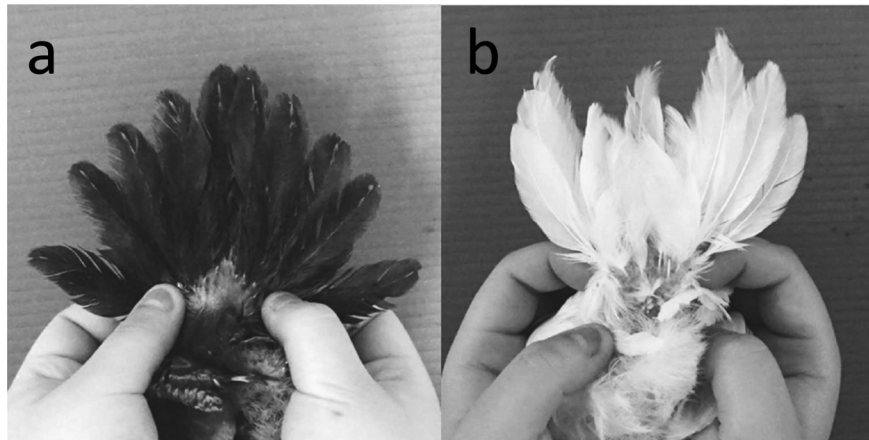


Fig. 4. Morphological difference in the shape of tail feathers. Measuring central and maximum tail lengths (C_TL and M_TL) revealed two types of tail feather shapes, fan-shaped tail feathers (a) and crescent-shaped tail feathers (b) in the growth process. Fan-shaped tail feathers indicate that the maximum tail was observed at the central tails. On the other hand, crescent-shaped tail feathers indicate that the maximum tail is observed at the left- and right-side tails. Photos were taken at 6 weeks of age.

reveal not only significant breed and sex differences in tail length but also variations in tail feather shape throughout the growth stages.

Tsudzuki (2003) reported that shank lengths of Chabo and Minohikichabo adults were shorter than those of other Japanese indigenous breeds, consistent with the observations of the current study. In the present study, positive correlations between body weight and shank length at several growth stages were consistently observed. Previous reports have indicated strong positive correlations between body weight and shank length (Chambers, 1990; Goto *et al.*, 2019b). Therefore, some common QTLs affect both body weight and shank length. Since meat-type chickens require higher performance in meat production, body weight and shank length are essential traits. As body weight increases, there is a higher risk of leg disorders in broiler chickens (Knowles *et al.*, 2008). Therefore, a shorter shank length is desirable for heavier chickens (Deeb and Lamont, 2002). Given that QTL genotypes regulating shank length but not body weight are known, we can genetically improve shank length regardless of body size in meat-type chickens. This will improve not only meat production but also health status (Tsudzuki *et al.*, 2007). A long-term divergent selection experiment was performed with three strains of high-, control-, and low-body weight groups in Japanese quails (Aggrey *et al.*, 2003). Body weight data were applied to the growth curve, and it was found that the influence of selection was affected differently by sex. Therefore, further studies are needed to estimate the growth curves for the traits measured, including body weight, shank length, and tail length in Tosa-jidori, Chabo, and Minohikichabo. Since there were significant breed effects in all traits, the QTL genotypes in these three breeds are likely to differ.

From the phenotypic correlations among traits measured in this study, no or weak negative correlations were observed between body size traits (BW and SL) and tail traits (C_TL, M_TL, and TN) in males and females. Even in the moderate to high correlations among traits with age, there is evidence of partial genetic independence of most traits at different ages; therefore, it is important to accumulate QTLs that differ over time for the same trait (Abasht *et al.*, 2009; Tuiskula-Haavisto *et al.*, 2011; Goto *et al.*, 2019b). Although the genetic architecture of tail traits is unresolved, there is likely to be a partial genetic independence between body size and tail phenotypes. The present results imply that there will be partially different genetic controls between body size and tail phenotypes. Osman *et al.* (2006) revealed pairwise genetic distances (D_A) among 34 breeds/populations of Japanese indigenous chickens and seven foreign breeds/varieties using 20 microsatellite markers. The D_A genetic distances ranged from 0.103 to 0.716 in 41 breeds/populations, with a mean of 0.463. Since the D_A values between Minohikichabo and Chabo (0.447), Minohikichabo and Tosa-jidori (0.436), and Chabo and Tosa-jidori (0.432) have been determined, there are substantial differences in the genetic background among these three breeds. Moreover, in the phylogenetic tree, Minohikichabo was grouped into Cluster I, which includes tail feather-rich-type breeds (*e.g.*, Onaga-dori, Shoukoku, and Toutenkou) whose males have a large amount of flowing tail feathers and saddle hackles (Osman *et al.*, 2006). Given that the genetic background of Minohikichabo is similar to that of Onaga-dori, Shoukoku, and Toutenkou, the Minohikichabo is potentially representative of rich-type tail feathers in Japanese indigenous breeds. Generally speaking, genetic cross experiments using smaller individuals are ideal because they can save rearing space and feeding costs. Since the

body sizes of Minohikichabo, Chabo, and Tosa-jidori (less than 1 kg BW in adult) are smaller than Onaga-dori, Shoukoku, and Toutenkou (around 1.3–2.2 kg BW in adult; Tsudzuki, 2003), future genetic experiments to further understand the genetic basis underlying tail feather richness will be effective using Minohikichabo, Chabo, and Tosa-jidori.

Many linkage analyses and genome-wide association studies have been performed to identify QTLs for several phenotypes in chickens. Linkage analysis has identified QTLs affecting comb traits in the F₂ population (Sun *et al.*, 2015). Genome-wide association studies have revealed candidate genes affecting growth traits in F₂ birds using a 60 K SNP chip (Gu *et al.*, 2011; Emrani *et al.*, 2017). Recently, population genomics has been used to identify candidate genes for several phenotypes (Goto and Tsudzuki, 2017). Using indigenous Chinese chicken breeds, Wang *et al.* (2017) identified candidate genes affecting rumplessness on the Z chromosome using population genomics. They conducted RNA-seq with feather follicles using wild-type and rumpless chickens to identify the causative variants (Wang *et al.*, 2017). Recently, Li *et al.* (2020) identified causal genes for feathered legs in chickens by combining classical linkage mapping using an experimental cross and high-resolution identical-by-descent mapping using whole-genome sequence data. In future studies, we will perform not only population genomics and linkage analysis but also gene expression analysis using feather follicles to reveal candidate genes affecting morphological traits in Japanese indigenous chickens using the present fundamental data on age-dependent changes.

In conclusion, this study revealed the growth process of morphological traits at several time points using three Japanese indigenous chicken breeds, Tosa-jidori, Chabo, and Minohikichabo. This will be a substantial step for understanding the genetic mechanisms of morphological traits, especially tail feather traits.

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Conflicts of Interest

The authors declare no conflicts of interest.

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