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Anatomy

Morphological variations of caudal skeleton between three chicken breeds

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ABSTRACT. Although fancy caudae are important traits for chicken breeds, factors associated with their morphological diversity are not fully understood. We analyzed the caudal skeleton of the Tosa-jidori, Chabo, and Minohikichabo breeds with wild-type, erect, and rich caudae, respectively. Five of six Tosa-jidori chickens had four caudal vertebrae, whereas all six Chabo and five of six Minohikichabo chickens had five. The angle of the apex pygostyli with respect to the margo cranialis was significantly larger and smaller in Chabo and Minohikichabo than Tosa-jidori chickens, respectively. These findings indicated that the caudal skeleton is one of important factors for forming the characteristic traits of chicken breeds.

KEYWORDS: bleached bone, computed tomography, fowl, pygostyle, tail feather

Chickens were domesticated from red jungle fowls (*Gallus gallus*) ~9,500 years ago [10], then many breeds were created through selection for the livestock industry and ornamental use [1]. Most of the ~50 Japanese indigenous chicken breeds are fancy, and have ornamental tail feathers [6]. Tail feathers are important traits in ornamental chickens; thus understanding how they form helps to clarify the diversity of chicken breeds.

We previously detailed the morphological traits of Japanese Old Type-Tosa (Tosa-jidori), Japanese Bantam (Chabo), and Japanese Tail Dragger (Minohikichabo) small breeds that have different characteristic caudae [4]. Tosa-jidori is one of the oldest ornamental breeds in Japan, the smallest among Japanese native chickens, and they have curved tail feathers [6]. As red jungle fowls also have curved tail feathers, this caudal trait is recognized as wild-type. On the other hand, it is generally known that the Chabo and Minohikichabo have erect and rich caudae, as shown in Fig. 1, respectively [6]. The word "chabo" in "Minohikichabo" means small chickens in Japan, not the Japanese Bantam companion. The length of the tail feathers is similar among these breeds at the young chick (age, <24 weeks) stage, whereas the tail feathers become significantly longer in Minohikichabo than in Tosa-jidori and Chabo chickens after 28 weeks of age [4]. In addition, Minohikichabo have far more tail feathers between the uropygial gland and the cloaca than the other two breeds [4]. These findings provided a morphological basis for differences between wild-type and rich, but not erect caudae.

The skeletal structures that form the caudal region comprise a pygostyle and several free caudal vertebrae. The avian pygostyle is a unique flattened bone formed by the fusion of several terminal caudal vertebrae [2, 11], and the shape varies depending on the species [9]. Although pygostyle segment fusion is incomplete in 3-months-old chickens, all ossified pygostyle vertebrae fuse completely by the age of 5 months [5]. The pygostyle is attached to the main muscles that move caudae and is the main skeletal base of the uropygium (the posterior part of the body where tail feathers grow) that reflects the vertical shape [8, 11]. Avian caudae can move within the range of free caudal vertebrae, the number of which considerably varies even within the same species [2]. Chickens can have four or five free caudal vertebrae even within the same strain (e.g. Mikawa) although five is typical [11].

Considering these facts, caudal skeletal structures might be important in the formation of the characteristic appearance of chicken caudae. Here, we analyzed the skeletal bones that form the caudae of Tosa-jidori, Chabo, and Minohikichabo using computed topography (CT) and assessed the morphology of bleached pygostyles.

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Fig. 1. Three chicken breeds analyzed.

MATERIALS AND METHODS

Animals

A total of 18 frozen decapitated adult chickens (age, >6 months; n=3 males and females of each breed) that had been disused were analyzed by CT, and pygostyles from 51 other decapitated adult chickens (age, >5 months; males and females, n=8 and n=5 Tosa-jidori; n=8 and n=9 Chabo; and n=11 and n=10 Minohikichabo) were bleached. The Animal Care and Use Committee of Obihiro University of Agriculture and Veterinary Medicine approved the experimental protocol of this study (approval number 21-11), which complied with the Regulations on the Management and Operation of Animal Experiments.

CT imaging

We acquired CT images of caudae using an Aquilion TSX-201A scanner (Toshiba Medical Systems Corp., Otawara, Japan) under the following conditions: 120 kV, 350 mA, and slice thickness, 0.5 mm. Imaging data stored in DICOM format were processed using RadiAnt DICOM Viewer [3]. We visualized skeletal structures using the Angio and Bones B/W modes of a 3D Volume Rendering function and the relationship between the pygostyle and the uropygium using the Bones and Skin 3 mode.

Bleaching process

Caudae were boiled, and then any remaining tissues and muscles attached to the skeleton were completely removed. The relationship between the pygostyle and the running direction of tail feathers was evaluated in a single central tail feather that was retained in several specimens. Pygostyle specimens were bleached in 10% H_2O_2, and evaluated in the lateral view.

Measuring the angle of the apex of pygostyles

A total of 69 pygostyles (18 CT and 51 bleached specimens) were evaluated by the following three steps; 1) drawing a line connecting the top and bottom edges of the margo cranialis, 2) drawing a line connecting the top edge of the margo cranialis and the top of the apex pygostyli, and 3) measuring the angle of these lines (Fig. 2). We determined this as the angle of the apex of pygostyles in this study.

Statistical analysis

The angle of the apex of pygostyles of three breeds was analyzed using one-way analysis of variance (ANOVA) followed by *post-hoc* Tukey-Kramer test. To evaluate the sex difference, the angle of males and females in each breed was analyzed using non-paired *t*-test. The value for each breed was indicated as mean ± standard deviation (SD).

RESULTS

The number of free caudal vertebrae

The three breeds of chickens (total, n=18) had four or five free caudal vertebrae (Fig. 3), and 5 (83%) of 6 Tosa-jidori chickens had four free caudal vertebrae (Fig. 3). On the other hand, all six Chabo and 5 (83%) of 6 Minohikichabo chickens had five free caudal vertebrae (Fig. 3).

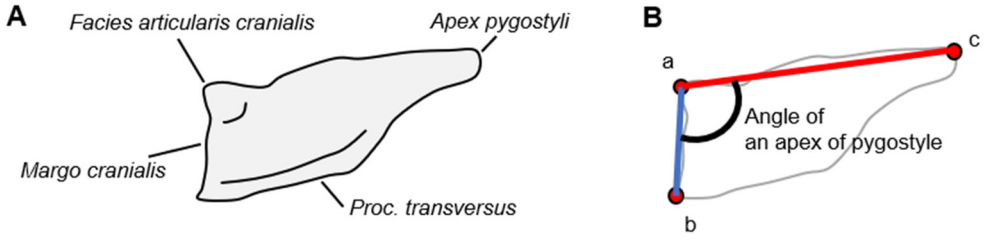

Fig. 2. Angle of an apex of pygostyle used in this study. (**A**) Schematic illustration of lateral view of a pygostyle. Left, anterior; upper, dorsal. (**B**) Angle of an apex of pygostyle (∠cab) analyzed in Fig. 5 corresponding to (A). a and b, top and bottom edges of margo cranialis; c, top of apex pygostyli.

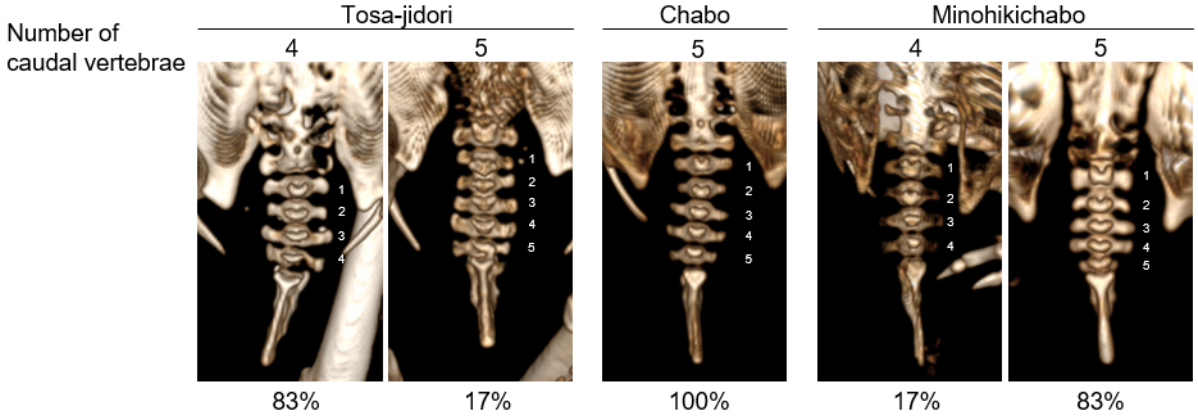

Fig. 3. Variations in numbers of caudal vertebrae. Representative reconstructed images of Tosa-jidori, Chabo, and Minohikichabo chickens. Ratios (%) are proportions of variations within each breed (n=6 in each).

Morphological variation of the pygostyles

The apex of the pygostyle reached the apex of the uropygium that was the basis of the running of the tail feathers in all three breeds (Fig. 4A). The angle of the apex of pygostyles varied among the three breeds ($P<0.001$), and that was significantly larger and smaller in Chabo (109.3 ± 5.8°) and Minohikichabo (87.9 ± 7.6°) than Tosa-jidori (101.8 ± 6.5°) chickens, respectively ($P<0.01$; Fig. 5). There were no differences in this angle between males and females in all three breeds ($P=0.62$, 0.74, and 0.34 in Tosa-jidori, Chabo, and Minohikichabo chickens, respectively; Fig. 5).

Pygostyles was divided into three types according to protrusion patterns; straight (in parallel with the processus transversus), upward, and downward (Fig. 4B). The bleached pygostyles with the central tail feather showed that the angle at which the tail feathers ran reflected the straight, upward, and downward slopes of the apex pygostyli (Fig. 4B). The straight, upward, and downward sloping pygostyles tended to present in the Tosa-jidori, Chabo, and Minohikichabo chickens, respectively (Supplementary Fig. 1).

DISCUSSION

The present CT findings revealed the variation of the number of free caudal vertebrae in chickens, indicating that the range of movement of free caudal vertebrae are prone to vary among breeds. Although most chickens including red jungle fowls (*Gallus gallus*) generally have five caudal vertebrae [11], most of 6 Tosa-jidori chickens, that had wild-type caudal characteristics like red jungle fowls, had four free caudal vertebrae. Because Tosa-jidori is the smallest among the Japanese native breeds [6], the fewer caudal vertebrae might be associated with miniaturization during domestication. In fact, some tailless mutants have been found among captive Tosa-jidori chickens [7], suggesting variety and a tendency for caudal vertebrae to disappear in this breed. On the other hand, Chabo and Minohikichabo chickens had mostly five free caudal vertebrae. This suggested that Chabo (erect caudae) and Minohikichabo (rich caudae) tended to have more caudal vertebrae than Tosa-jidori (wild-type caudae). As the uropygium moves within the range of movement of caudal vertebrae, the caudae including the tail feathers of Chabo and Minohikichabo seemed to have a greater range of motion than Tosa-jidori judging from the skeletal structures.

The present bleaching pygostyles indicated that the apex of pygostyle is the skeletal foundation of the directions in which the tail feathers ran. Therefore, we adopted the angle of the apex with respect to the margo cranialis as an indicator of pygostyle morphological features associated with the tail traits. This angle was maximum and minimum in Chabo with erect cauda and Minohikichabo chickens with rich cauda, respectively, indicating that the high and low angles of the apex of pygostyles confer advantages for the formation

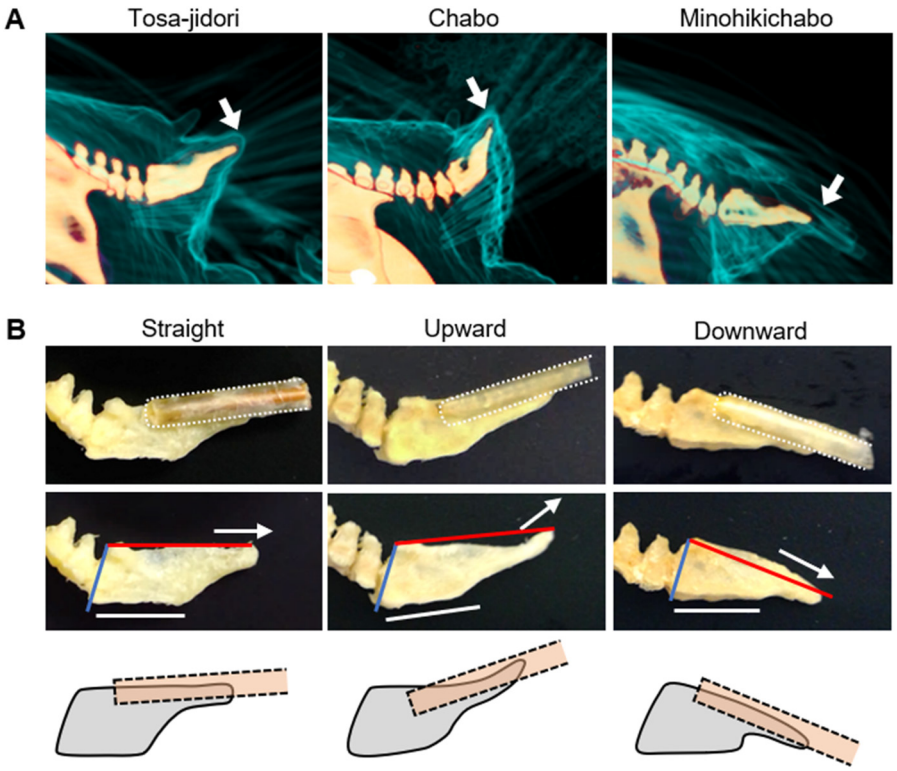

Fig. 4. Pygostyle forms uropygium base in three breeds. (**A**) Representative reconstructed computed tomography (CT) images of caudae. Cyan, body surface. Arrows, uropygium apex. (**B**) Straight-, upward-, and downward-protruding pygostyles. Representative images of bleached pygostyles with (upper) and without (middle) central tail feathers (dashed lines). White bars and arrows indicate baselines of processus transversus and directions of apex pygostyli. Blue and red lines are corresponding to those in Fig. 2B. Schematic illustrations (lower) summarize a relationship between shape of pygostyles (gray) and central tail feathers (orange).

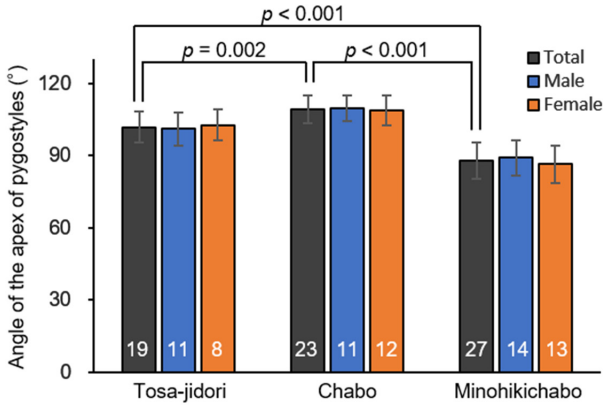

Fig. 5. Angle of an apex of pygostyle in three breeds. This angle of a total chickens (gray bars) varies among three breeds significantly (P<0.001, one-way ANOVA), and that is larger and smaller in Chabo and Minohikichabo than Tosa-jidori chickens, respectively (P<0.01, Tuckey-Kramer test). There are no significant differences between males (blue bars) and females (orange bars) in each breed (P>0.1, non-paired t-test in each). Number in bars indicates a number of specimens analyzed in each group. Mean ± SD.

of erect and rich caudae, respectively. In addition, tendency of the sloping (straight in Tosa-jidori, upward in Chabo, and downward in Minohikichabo) was corresponded with each angle of the apex of pygostyles in three breeds. These findings suggested that each morphological feature of the pygostyles has been preserved in Chabo and Minohikichabo chickens after selective breeding.

The present findings revealed that the skeletal morphology of caudal structures varies among chicken breeds, and that this helps to form the characteristic appearance of chicken caudae (Fig. 1). Chabo and Minohikichabo chickens with characteristic tails apparently have a larger range of tail motion than Tosa-jidori chickens with wild-type tails, and this depends on the number of free caudal

vertebrae. In addition, the shape of pygostyles in Chabo and Minohikichabo also has an advantage in terms of the running angle of tail feathers. The caudal skeleton might be a representative example of variable traits that have been conserved during breeding processes. However, it remains still unclear whether these morphological features apply to other breeds and how they are formed during developmental stages. In addition to approaches for these questions, further studies of muscles associated with tail motion, such as levator, lateralis, and depressor caudae, might reveal differences in caudae among breeds in more detail from the viewpoint of the musculoskeletal system.

CONFLICT OF INTEREST. The authors declare no conflict of interest.

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