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S. Struthers,[*,†,1] B. Andersson,[‡] M. Schmutz,[‡] H. A. McCormack,[*] P. W. Wilson,[*] I. C. Dunn,[*] V. Sandilands,[†] and J. J. Schoenebeck[*]

[*]*The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, EH25 9RG UK;* [†]*Department of Agriculture, Horticulture, and Engineering Science, Scotland's Rural College, Edinburgh, EH25 9RG UK; and* [‡]*Lohmann Breeders GmbH, Cuxhaven, 27472, Germany*

ABSTRACT Beak treatment is an effective method of reducing the damage inflicted by severe feather pecking (**SFP**) but there is significant pressure to eliminate these treatments and rely solely on alternative strategies. Substantial variation in beak shape exists within non-beak treated layer flocks and beak shape appears to be heritable. There is the potential to use this pre-existing variation and genetically select for hens whose beak shapes are less apt to cause damage during SFP. To do this, we must first understand the range of phenotypes that exist for both the external beak shape and the bones that provide its structure. The objective of this study was to determine the variation in premaxillary (within the top beak) and dentary (within the bottom beak) bone morphology that exists in 2 non-beak treated pure White Leghorn layer lines using geometric morphometrics to analyze radiographs. Lateral head radiographs were taken of 825 hens and the premaxillary and dentary bones were landmarked. Landmark coordinates were standardized by Procrustes superimposition and the covariation was analyzed by principal components analysis and multivariate regression using Geomorph (an R package). Three principal components (**PCs**) explained 85% of total premaxillary bone shape variation and showed that the shape ranged from long and narrow with pointed bone tips to short and wide with more curved tips. Two PCs explained 81% of total dentary bone shape variation. PC1 described the dentary bone length and width and PC2 explained the angle between the bone tip and its articular process. For both bones, shape was significantly associated with bone size and differed significantly between the two lines. Bone size accounted for 42% of the total shape variation for both bones. Together, the results showed a range of phenotypic variation in premaxillary and dentary bone shape, which in turn may influence beak shape. These bone phenotypes will guide further quantitative genetic and behavioral analyses that will help identify which beaks shapes cause the least damage when birds engage in SFP.

Key words: Lohmann, principal components analysis, radiography, bone shape, laying hen

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INTRODUCTION

Animal welfare and how food production animals are raised and managed are becoming more important to consumers and society in general (Napolitano et al., 2010; Spooner et al., 2014; Dunne and Siettou, 2020). This concern for animal welfare has led to widespread legislation regarding how animals are housed (Centner, 2010). In the egg production industry, the use of alternative housing systems such as free-range or whole-barn housing has increased, largely in response to the shift away from and/or ban on cages (either conventional or furnished) in many countries. For example, 56% of the 11 billion eggs produced in the United Kingdom (**UK**) in 2019 came from free-range systems (British Egg Industry Council, 2019). In the United States, over 29% of laying hens are housed in cage-free systems, marking a 15% increase since 2016 (United Egg Producers, 2021). These alternative systems allow birds more space and the ability to express their full behavioral repertoire (Muir et al., 2014). However, the large group sizes and unstable social hierarchies in these systems can contribute to outbreaks of severe feather pecking (**SFP**) (Bilčík and Keeling, 2000; Lay et al., 2011; Muir et al., 2014). SFP is the pecking at, pulling, and

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[1]Corresponding author: sarah.struthers@ed.ac.uk

removal of feathers which can cause damage to the integument of the birds, often resulting in wounds and cannibalism (Savory, 1995). The behavior is a serious welfare and economic concern as the loss of feathers can result not only in pain and mortality but in increased feed costs and poor feed efficiency (Leeson and Walsh, 2004).

The most effective method to control SFP is beak treatment, which both blunts and shortens beak length. Whether by hot blade trimming or infrared methods, beak treatment raises its own welfare concerns. Hot blade trimming results in acute pain and may cause neuroma formation and chronic pain, depending on the age of the bird at trimming and the severity of the trim (Lunam et al., 1996; Gentle et al., 1997). Infrared beak treatment has much less of a negative impact on bird welfare than hot blade trimming (Gentle and McKeegan, 2007; Dennis et al., 2009; Struthers et al., 2019) but concern regarding any form of beak manipulation has led to many national governments banning these treatments or, such as in the United Kingdom, allowing them only until better SFP prevention methods are found (Department for Environment Food and Rural, 2010; Scottish, 2010).

Alternative management practices to beak treatment have been studied and led to some successes in reducing the incidence of SFP; however, the behavior remains unpredictable and difficult to control (Jendral and Robinson, 2004). More recently, there has been an increased focus on selective breeding against SFP (Rodenburg et al., 2003; Bennewitz et al., 2014; Grams et al., 2015). The incorporation of meaningful behavioral data into a breeding program can be challenging; therefore, quantifiable outcomes such as plumage cover, liveability, and beak shape are measured (Ellen et al., 2019). Plumage cover has been reported to have a moderate heritability and selection for the trait has reduced the incidence of SFP (Brinker et al., 2014; Icken et al., 2017). However, a disadvantage is that only the victims of feather pecking can be detected and it is time consuming to measure (Brinker et al., 2014). A hen's liveability in relation to feather pecking depends both on her ability to avoid being feather pecked and the tendency of her flock mates to feather peck (Ellen and Bijma, 2019). Group selection for liveability reduced beak-related injuries and mortality in non-beak treated laying hens (Kuo et al., 1991; Craig and Muir, 1993; Rodenburg et al., 2010; Ellen and Bijma, 2019) but continual selection caused heritability to decrease over time meaning that further improvement becomes more and more difficult (Ellen and Bijma, 2019).

Beak shape differs between bird species because each species has evolved a specific shape to fit both their diet and environmental niche (Grant and Grant, 1993). The beak is a highly specialized and complex organ that serves many important functions including feeding, drinking, grooming, parasite removal, and defense (Lunam, 2005). In chickens, the natural shape of the beak resembles that of a hook, with the sharp tip of the top beak extending over the bottom beak. The internal structure of the top and bottom beak is provided by the premaxillary and dentary bones, respectively (Lunam, 2005). To what extent beak shape can contribute towards reducing the incidence of SFP is still not fully understood. It is also not well understood if phenotypic variation in beak shape is due primarily to genetics, environmental factors, or a combination of both. Substantial variation in beak shape exists within non-beak treated layer flocks and beak shape appears to be heritable (Icken et al., 2017). Using a device that measured the difference in length between the top and bottom beak (i.e., top beak overhang), Icken et al. (2017) found that when hens had naturally shorter (blunter) top beaks, mortality was reduced and plumage cover improved. This suggests that there is the potential to use this pre-existing variation and selectively breed hens whose beak shapes are less apt to cause damage during SFP.

Beak morphology in poultry has previously been described using linear measurements such as length, width, and depth (Marchant-Forde et al., 2008; Carruthers et al., 2012; McKeegan and Philbey, 2012). However, these measurements help highlight differences in size rather than true shape differences. Interpretation of these measurements is also difficult when the study specimen occupies three-dimensional space. Geometric morphometrics is the analysis of morphological shape using landmark coordinates rather than linear measurements (Zelditch et al., 2012). Geometric morphometrics is typically applied to dead specimens (museum specimens, field collections preserved in fixative) or if done using live animals, the method of image acquisition for two-dimensional (**2D**) analysis is often photography.

Dalton et al. (2017) investigated the effects of age, sex, and beak size on beak shape variation in domestic turkeys using photography and geometric morphometrics. The authors reported a wide range of phenotypic variation in turkey beak shape with sex and beak size having a much greater effect on shape than age. The shape patterns reported by Dalton et al. (2017) are similar to those found in other bird species (Foster et al., 2008; Kulemeyer et al., 2009; Shao et al., 2016). Although they did not look at the beak specifically, Stange et al. (2018) compared skull morphology between domesticated chicken breeds and wild fowl using geometric morphometrics analysis of microscribe-acquired landmark coordinates. Domesticated chickens occupied a greater portion of morphospace (shape space) and therefore had greater variation in skull shape compared to their wild fowl ancestors (Stange et al., 2018).

The objective of this study was to radiograph breeding stock consisting of 2 different pure lines of White Leghorn hens to 1) generate bone morphometric data and 2) describe the phenotypic variation in premaxillary (within the top beak) and dentary (within the bottom beak) bone shape that exists within these two populations. The collection of these phenotypes will allow for the calculation of genetic parameters (heritability, QTL, and estimated breeding values) in future studies. As

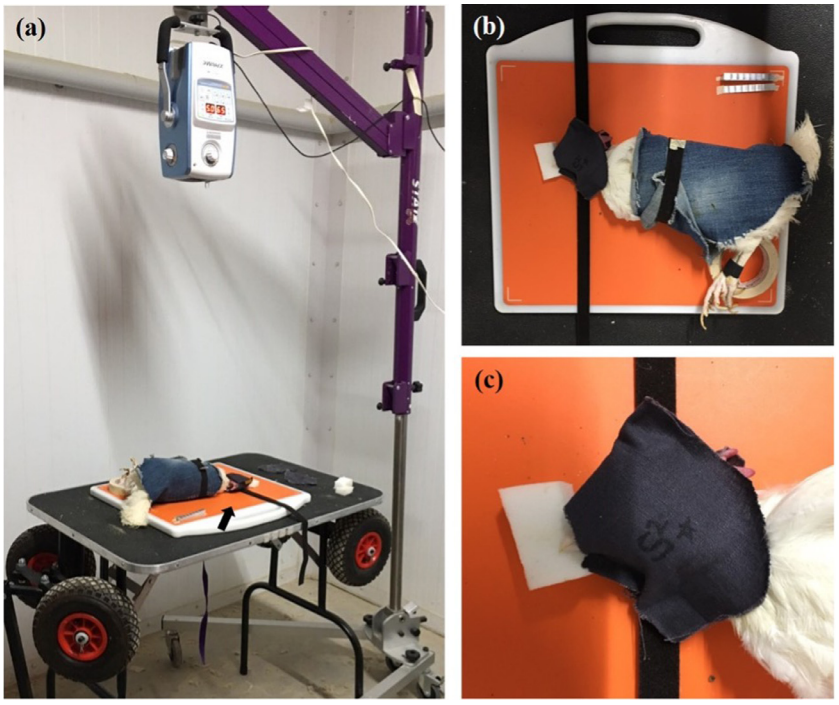

Figure 1. (A) Set up of the portable radiograph device on the laying hen farm. Arrow indicates radiograph plate. Body (B) and head (C) restraint devices used during radiograph acquisition.

mentioned, geometric morphometrics is typically applied to dead specimens or if using live specimens, applied to photographs. To our knowledge, geometric morphometrics has not been applied to radiographs for the assessment and characterization of beak morphology in poultry. Radiography offers the advantage of being able to analyze the bones, which may contribute to overall beak shape and size. This study helped develop the novel capability of using radiography for the application of geometric morphometrics in live, nonsedated laying hens.

MATERIALS AND METHODS

This study was reviewed and approved by the Animal Welfare and Ethical Review Body at the Roslin Institute (University of Edinburgh). This study was conducted in the United Kingdom under a Home Office project license (70/7909) and complied with UK regulations regarding the treatment of experimental animals (Home Office (UK), 2014).

Animals and Housing

Birds (n = 825) used for this study were from 2 pure line populations (Line A and B) of White Leghorn laying hens. Both Line A (n = 387) and Line B (n = 432) hens contribute to the four-line breeding scheme of Lohmann Selected Leghorn (**LSL**) hybrid layers (Lohmann Breeders GmbH, Cuxhaven, Germany). Hens from both populations were housed in conventional cages (n = 200) in family groups (full-siblings and/or half-siblings) with a maximum of 5 birds per cage.

Radiographic Examination

Equipment The radiograph device used was a MeX +20BT Lite portable x-ray generator with a Cuattro Slate 6DR system (Figure 1A). The generator was suspended over the radiograph plate and a 2-m controlled radiation zone was demarcated using plastic chain-link barriers. A lead curtain separated the controlled radiation zone from the bird handling zone.

To reduce handlers' exposure to radiation, custom restraint devices were developed to hold the hens in place. The restraint was performed using methods adapted for veterinary radiograph examination. A restraint jacket was made of cotton denim. Birds were wrapped in the cotton jacket, which was then secured by an attached strap of hook and loop tape (Figure 1B). A separate strap of hook and loop tape was used to secure the legs together. For immobilizing the head, a cotton hood was custom designed (Figure 1C). The hood could be placed over the head and the beak would show through a small opening at one corner. The hood was secured underneath the head by small hook and loop fasteners. These could be adjusted to accommodate different sized heads and combs. Once the hen was placed in right lateral recumbence on the radiograph plate, the cloth hood was secured to a strap of hook and loop tape running along the radiograph plate. For optimal positioning, a small piece of radiolucent foam was placed underneath the beak for the duration of the radiograph.

Procedure All hens from 2 cages were removed from their home cage, loaded into an enclosed transport trolley, and transported to the radiation zone (up to 10 hens per transport trolley). Each side of the trolley had 5 smaller cages in which one hen were placed and each home cage took up one side of the trolley. Hens were

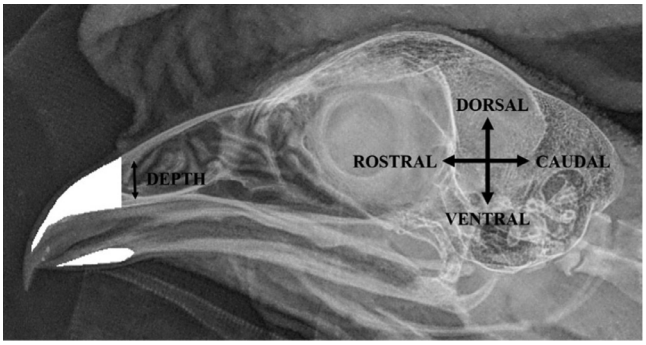

Figure 2. Schematic demonstrating the anatomical terms of location used to describe premaxillary (within the top beak) and dentary (within the bottom beak) bone shape. The anatomical terms of location are in relation to the white shaded area on each bone.

removed from the trolley one a time, had their wing band recorded, and then were restrained as described above. The handlers left the radiation zone and a lateral radiograph was taken. The hen remained conscious and nonsedated for the entire procedure. The radiograph was checked for image quality (e.g., blurriness). If the radiograph was not usable, another was taken. After verification of a usable radiograph, the hen was lifted off the radiograph plate, the fabric hood and jacket were removed, and the hen was immediately returned to the trolley where it remained until the procedure was complete for all hens in the trolley. When returning the hens to their home cage, the wing band numbers belonging to each cage were verified by matching them to a sticker on the front of the cage. Following catching and placement in the transport trolley, the entire procedure lasted approximately 120 s for each hen, beginning with removal from the trolley, restraint, radiograph acquisition, and return to the trolley. A total of 825 radiographs (one per hen) were collected over 9 d.

Radiograph Analysis

Formatting Left lateral radiographs (DICOM format) were cropped to 600 × 600 pixels and uniformly sharpened (Sharpen feature) using ImageJ analysis software (v.1.53g; National Institutes of Health, Bethesda, MD). Radiographs were cropped to show the entire head and the C1 vertebra. Radiographs were excluded from landmarking if the image was blurry or if the landmarks could not be properly placed on their locations. This resulted in 819 and 710 lateral radiographs being available for premaxillary and dentary landmarking, respectively. The difference in the number of radiographs available for premaxillary vs. dentary landmarking was primarily due to blurriness from the hen moving her lower mandible during radiograph acquisition.

Placement of Landmark Coordinates The radiographs were landmarked in ImageJ using the Multipoint Tool. The anatomical terms of location used to describe the placement of the landmarks and the shape of both bones are provided in Figure 2. Thirteen landmarks (**LMs**) were chosen for the premaxillary and dentary bones (Figure 3). LMs 1 to 3 were placed on locations that had the same relative position (homology) between specimens (Zelditch et al., 2012). LMs 4 to 13 consisted of sliding semilandmarks and helped quantify the shape of the bone (Gunz and Mitteroecker, 2013). These LMs slid along the curves of the bones until their positions were optimized to minimize shape differences (Gunz and Mitteroecker, 2013). The 2D landmark coordinates were copied from the Results table in ImageJ into a Microsoft Excel spreadsheet. The spreadsheet (.xlsx format) was imported into R (v.3.6.1) and then R-based scripting and data visualization were done using the integrated development environment RStudio (v.1.2.5001) (R Core Team, 2019).

Geometric Morphometric Analysis Multivariate shape analysis was done using the R package Geomorph (v.4.0.0) (Adams et al., 2021) . Outliers in the upper quartile as shown by Procrustes distance to the mean (square root of the sum of squared distances between the individual shape measurement and the consensus shape) were removed from analysis; this reduced the dataset of 2D landmark coordinates to 806 premaxillary and 684 dentary lateral radiographs. Using Procrustes superimposition, the 2D landmark coordinates for all the radiographs (each bone separately) were standardized such that landmarks were superimposed, re-scaled, and rotated to a common orientation. The resulting transformed landmarks are termed Procrustes coordinates. A distance matrix of individuals was calculated from the Procrustes coordinates. The covariation described by the matrix was decomposed by principal components analysis (**PCA**) and eigenvectors and their

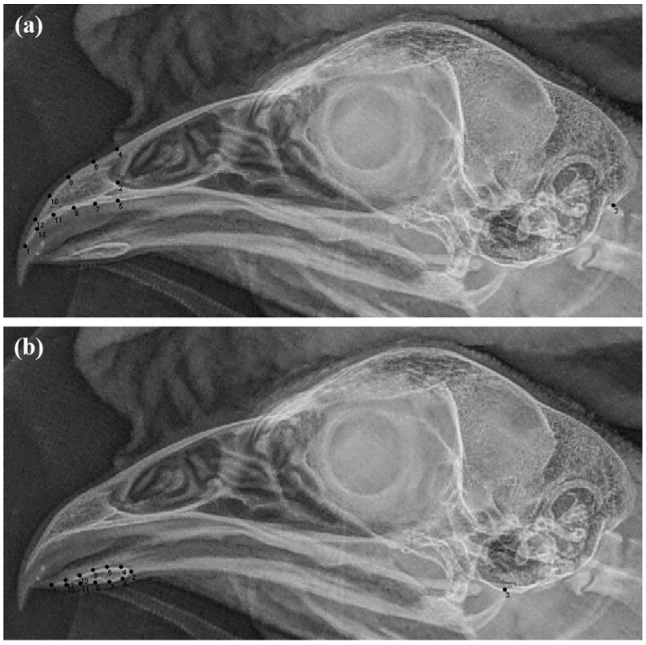

Figure 3. The landmarks (LMs) and semilandmarks used for the analyses of the left lateral radiographs. (A) premaxillary bone (LM 1, tip of premaxillary bone; LM 2, rostral end of the nares; LM 3, base of skull where spinal cord enters; LMs 4−13, semilandmarks) and (B) dentary bone (LM 1, rostral tip of dentary bone; LM 2, caudal-most end of dentary bone; LM 3, caudal-most end of angular bone; LMs 4−13, semilandmarks).

corresponding eigenvalues were found. The eigenvector with the highest eigenvalue was the first principal component (**PC**) and explained the most covariation in shape. Each sequential PC (eigenvector) described continuously smaller covariation (eigenvalue). These eigenvalues allowed the shape variables to be ordered in morphospace. From there, similarities and differences in shape were interpreted from where each individual point (corresponding to 1 hen) was on the PCA plot.

The Procrustes superimposition also created a consensus bone shape by identifying the centroid (center point) of all the landmarks and semilandmarks in each radiograph. The centroid size was then calculated as the square root of the sum of squared distances of landmarks from the centroid (Zelditch et al., 2012). In the present study, centroid size served as a proxy for relative bone size.

Statistical Analysis To test for allometry (the influence of size on shape) for each bone, a multivariate regression of the Procrustes shape coordinates onto log centroid size for the entire dataset was performed. A Procrustes ANOVA was performed to determine if bone shape variation and allometry differed between the 2 pure lines. Data were analyzed using the procD.lm function within Geomorph. Significance was evaluated with a residual randomization permutation procedure with 1,000 iterations. A Spearman correlation using the cor.test function in the R Stats package was performed to test the relationship between 1) PC1 scores of the premaxillary and dentary bones (i.e., the relationship between the two bone shapes) and 2) log centroid sizes of the premaxillary and dentary bones (i.e., the relationship between the two bone sizes). Differences were considered significant when $P \leq 0.05$.

RESULTS

Premaxillary Bone

For the premaxillary bone, there were 3 principal components that explained the majority of the total variation in premaxillary bone shape in the 2 populations of pure line hens. PC1 accounted for 56% of the total variation. Though the two lines overlapped in morphospace, there was noticeable separation between them. In contrast to Line A, Line B tended toward premaxillary shapes with more pronounced downward curvature. Also, the caudal end of the premaxillary bone tended to

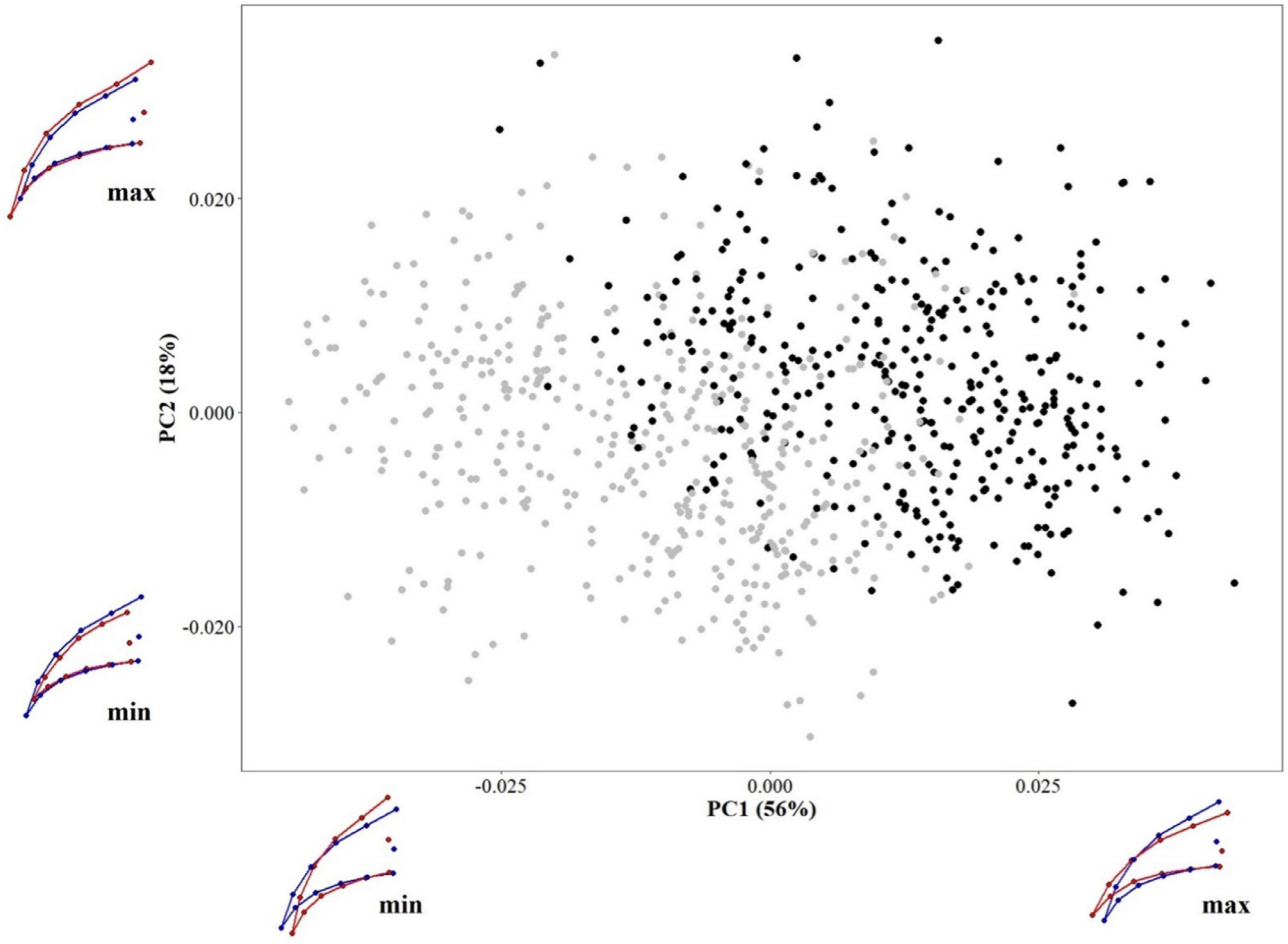

Figure 4. The premaxillary bone shape variation explained by PC1 and PC2 for Line A (black) and B (gray) pure line hens. The point figures at each end of the x and y axes are visual representations of the premaxillary bone shape. The blue lines represent the mean shape of the premaxillary bone for the two lines. The red lines represent the premaxillary bone shape at the minimum and maximum values along the axes of PC1 and PC2.

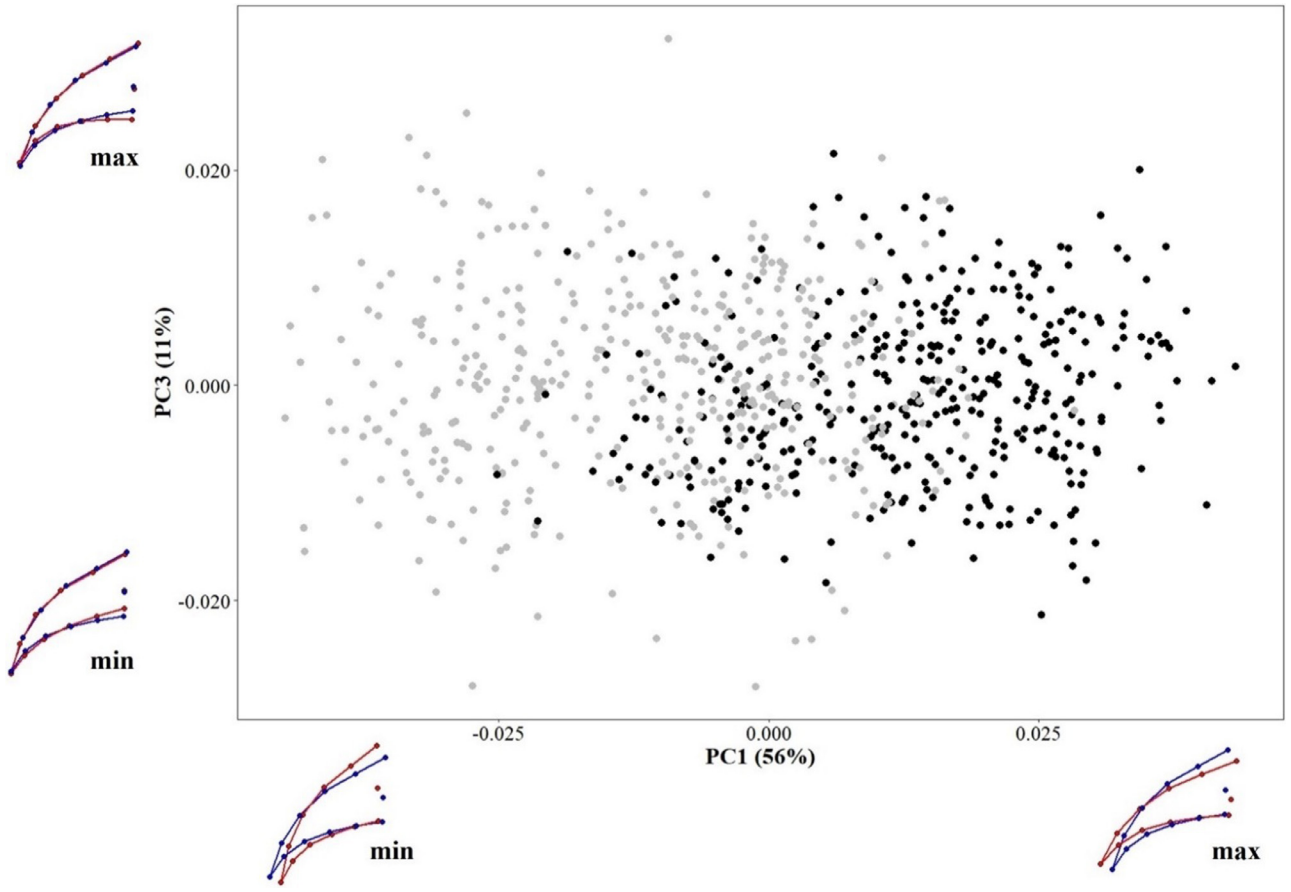

Figure 5. The premaxillary bone shape variation explained by PC1 and PC3 for Line A (black) and B (gray) pure line hens. The point figures at each end of the x and y axes are visual representations of the premaxillary bone shape. The blue lines represent the mean shape of the premaxillary bone for the two lines. The red lines represent the premaxillary bone shape at the minimum and maximum values along the axes of PC1 and PC3.

be deeper in Line B (Figure 4). PC2 accounted for 18% of the total shape variation and describes a rostral (PC2 min) vs. caudal (PC2 max) shift in the dorsal and ventral margins of the bone and a corresponding caudal (PC2 min) vs. rostral (PC2 max) shift of the bone tip (Figure 4). PC3 explained 11% of the total shape variation and described a rostral (PC3 min) vs. caudal (PC3 max) shift in the dorsal/ventral beak margins as well as a slight narrowing (PC3 min) vs. widening (PC3 max) of the caudal-most ventral margins of the premaxillary bone (Figure 5).

To test whether there was a relationship between bone size and its shape (static allometry), the Procrustes shape coordinates for the entire dataset were regressed on log centroid size. The regression revealed that the shape coordinates were significantly associated with bone size (Z-score = 10, $P < 0.01$) and that bone size accounted for 42% of the total shape variation. Based on Procrustes ANOVA, premaxillary bone shape also differed significantly between the two lines (Z-score = 8, $P < 0.01$). Allometry was also unique between the lines as the interaction between log centroid size and line was significant ($P = 0.04$). Line A hens had larger log centroid sizes (mean = 6.11 ± 0.002) compared to Line B (mean = 6.04 ± 0.003). Because this interaction was significant, no attempt was made to correct for allometry.

Dentary Bone

The PCA of the dentary bone revealed 2 principal components that explained 81% of the total shape variation in the two lines. PC1 accounted for 62% of the shape variation and described a widening and lengthening (PC1 min) vs. a narrowing and shortening (PC1 max) of the bone (Figure 6). As observed with the premaxillary bone, PC1 appeared to separate the lines, with a longer/wider dentary bone more characteristic of Line B. PC2 accounted for 19% of the total variation and described the angle between the dentary bone and its articular process associated with an inferior (PC2 min) vs. superior (PC2 max) shift of the caudal end of the dentary bone and a superior (PC2 min) vs. inferior (PC2 max) shift of the bone tip (Figure 6).

Multivariate regression of the Procrustes shape coordinates on log centroid size for the entire dataset showed that dentary bone shape was significantly associated with its size (Z-score = 8, $P < 0.01$) and that bone size accounted for 42% of the total dentary shape variation. Like the premaxillary bone, dentary bone shape differed significantly between lines (Z-score = 7, $P < 0.01$) as did allometry ($P < 0.01$). Line A had larger log centroid sizes (mean = 5.81 ± 0.005) compared to Line B (mean = 5.68 ± 0.007). As with the premaxillary bone,

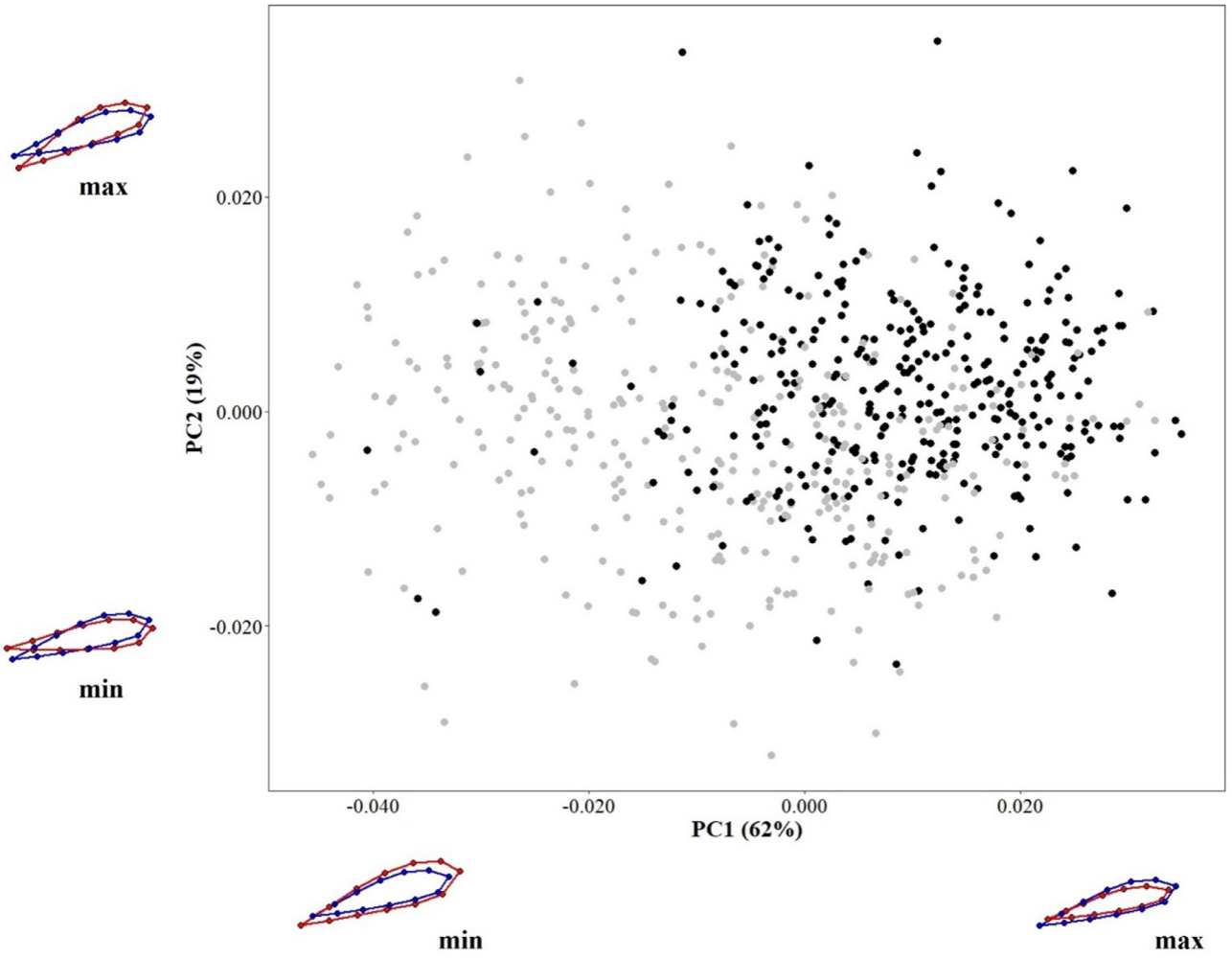

Figure 6. The dentary bone shape variation explained by PC1 and PC2 for Line A (black) and B (gray) pure line hens. The point figures at each end of the x and y axes are visual representations of the dentary bone shape. The blue lines represent the mean shape of the dentary bone for the two lines. The red lines represent the dentary bone shape at the minimum and maximum values along the axes of PC1 and PC2.

the interaction between log centroid size and line was significant for the dentary bone ($P < 0.01$) so no attempt to correct for allometry was made.

Spearman Correlation

Correlation between the PC1 scores of the premaxillary and dentary bones found that there was a strong, positive relationship ($r_s = 0.69$; $P < 0.01$) between the shapes of the premaxillary and dentary bones. Using log centroid size as a proxy for bone size, a strong, positive relationship between the sizes of the premaxillary and dentary bones was also found ($r_s = 0.96$, $P < 0.01$).

DISCUSSION

Many previous studies examining skull and beak morphology in different avian species have done so from an evolutionary and developmental perspective (Foster et al., 2008; Bright et al., 2016, 2019; Shao et al., 2016; Cheng et al., 2017), focusing on how the beak and skull have morphologically evolved in response to each species' particular ecological niche. This differs from the present study whose aim is to understand the underlying shape of the bones within the beak so that it potentially can be used as a tool to help improve management of non-beak treated laying hen flocks. To our knowledge, this is the first study to analyze and describe the morphology of the bones within chicken beaks using radiography and landmark-based geometric morphometrics. As mentioned previously, geometric morphometric techniques are typically used on dead specimens. A challenge to collecting beak morphology data (phenotypes) for use in further genomic and behavioral studies is collecting it in live animals. Geometric morphometrics has been applied to photographs in live turkeys (Dalton et al., 2017); however, photography does not allow for the analysis of bone shape. Radiography offers the advantage of being able to analyze both beak and bone shape but there are technical challenges with collecting radiograph data from live, nonsedated birds as birds need to be restrained during x-ray capture to limit bird and human exposure to radiation. This study showed that meaningful phenotype data can be collected from live laying hens using the methods described.

The present study found that premaxillary bone shape variability explained by PC1 and PC2 ranged from long,

narrow bones with pointed tips to short, wide bones with more curved tips. Variability in dentary bone shape explained by PC1 and PC2 ranged from short and narrow to long and wide with corresponding superior vs. inferior shifts of the bone tip. The closest related study to the present one would be that of Dalton et al. (2017) who examined the external beak shape of domestic turkeys. The authors found that top beak shape in domestic turkeys ranged from short, narrow beaks with short, pointed beak tips to long, wide beaks with long, curved beak tips. Bottom beak shape in turkeys ranged from wide and round to narrow and thin with corresponding shifts in the beak tip (Dalton et al., 2017).

The axes described by both PC1 and PC2 for premaxillary bone shape variation in this study are similar to top beak shapes reported previously in other avian species: long and narrow vs. short and wide (Foster et al., 2008; Kulemeyer et al., 2009; Shao et al., 2016; Dalton et al., 2017). Although it is presumed that beak shape reflects the underlying structures of the premaxillary and dentary bones, we are unaware of any study that has formally tested this. Thus, caution is required when comparing our results to those whose analyses are based on beak shape. Regarding the curvature of the premaxillary bone tip, the present study found that hens with long and narrow premaxillary bones had more pointed (less curved) bone tips while hens with short and wide premaxillary bones had more curved (less pointed) bone tips. In the present study it is difficult to determine which bone tip shape (pointed vs. more curved) results in a sharper external beak, particularly since there is often keratin growth extending beyond the tip of the premaxillary bone which could affect the curvature. The two lines were partially separated by PC1 for the premaxillary bone, which suggests that there are distinct bone phenotypes (particularly regarding the curvature and allometry) within each genetic line. This information is important as the incorporation of multivariate shape data into selection indices could help guide the selection of hens whose beak shapes are less apt to cause damage during SFP.

There were also mild similarities in the dentary bone shapes described in the present study and the bottom beak shapes reported by Dalton et al. (2017). In both studies, shape variability described by PC1 and PC2 could be interpreted as a widening vs. narrowing of the bone or beak with corresponding superior vs. inferior shifts of the bone or beak tip. However, once again these comparisons should be made with caution. Here, bone size accounted for approximately 42% of both the premaxillary and dentary bone shape. This is similar to what has been reported in raptors (Bright et al., 2016) and for the bottom beak of domestic turkeys (Dalton et al., 2017) and suggests that bone shape is strongly influenced by bone size. However, more research investigating the relationship between bone shape and size and the influence of body size is needed. Further studies could also incorporate lateral landmarks on different locations of the skull to determine the relationship between skull and beak shape and size. Beak and skull shape in avian species are highly integrated, meaning that change in one correlates to change in the other (Bright et al., 2016; Stange et al., 2018). It is still not fully understood to what magnitude skull shape impacts beak shape and how size factors in, although Stange et al. (2018) reported a weak effect of skull size on shape in domestic chicken breeds.

The positive correlations for both the PC1 scores and the log centroid sizes across both lines suggest a coordination of the growth and subsequent shape between the bones (i.e., the dentary bone grows and is shaped in relation to the premaxillary). This coordination may be explained by the developmental origins of beak morphology. Cranial neural crest cells contain the patterning information responsible for beak morphology variation between different avian species and both the premaxillary and dentary bone are derived from these cells (Schneider and Helms, 2003). Cranial neural crest cells may regulate the development and growth of the dentary bone such that it always fits inside the premaxillary and in fact, the top beak does contain a groove that the bottom beak fits in to (also known as the bearing horn) (Lucas, 1972). However, to our knowledge, this has yet to be formally verified and because of this, caution should be used when interpreting the significant correlations as biologically important.

The results of this study demonstrate significant phenotypic variation in the shape of both the premaxillary and dentary bone within 2 populations of pure line White Leghorn laying hens. The present study is the first in a series that will investigate the impact of beak shape on feather pecking-related damage in laying hens. The aim of this study was to identify various bone phenotypes as an initial step rather than to associate a certain phenotype with damage. Research into why hens feather peck and other methods of mitigating the behavior is ongoing and the present study helps contribute to that knowledge by laying the foundation to investigate if the beak itself can be used as a mitigation tool. Subsequent morphometric analyses of these two hen populations will analyze the external shape of the beak and help elucidate the relationship between bone and beak shape and its impact on SFP behavior, plumage cover, and mortality. These bone and beak phenotypes will also be used in quantitative genetic analyses for the calculation of beak and bone shape heritability and to identify QTL underlying beak morphology.

DISCLOSURES

The authors declare that they have no competing interests except for SS who received financial support from Lohmann Breeders and BA and MS who are employees of Lohmann Breeders.

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