An analysis of the maxillary beak shape variation between 2 pure layer lines and its relationship to the underlying premaxillary bone, feather cover, and mortality

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ABSTRACT Beak shape varies considerably within and between intact-beak laying hens, and aspects of beak shape appear to be heritable. As an alternative to beak treatment (an effective method of reducing damage from severe feather pecking (SFP)), this variation could be used to genetically select hens whose beak shapes are less apt to cause damage. To be able to select certain phenotypes, the beak shape variation that exists within laying hen flocks must first be characterized. The objectives of this study were to 1) describe the maxillary beak shape variation in 2 pure White Leghorn layer lines with intact beaks using geometric morphometrics to analyze images, and 2) examine the beak shape’s relationship to the premaxillary bone, feather cover, and mortality. A lateral head image was taken of each hen (n = 710), and 20 landmarks were placed along each image’s dorsal and ventral margins of the maxillary beak. Landmark coordinates were standardized by Procrustes superimposition, and the covariation was analyzed by principal components analysis and multivariate regression. Feather cover was scored at 3 ages and mortality was monitored throughout the production cycle. Three principal components (PCs) explained 83% of the maxillary beak shape variation and the first PC partially separated the 2 lines. Maxillary beak shapes ranged from long and narrow with pointed tips to short and wide with more curved tips. Moderate correlations were found between the maxillary beak and premaxillary bone shape ($r_s = 0.44$) and size ($r_s = 0.52$). Line A hens had better feather cover than Line B at all ages. Line A hens also had less total and cannibalism-related mortality than Line B (10.7 and 0.4% vs. 16.7 and 2.4%, respectively). Beak shape may be one factor contributing to the observed differences in feather cover and mortality. The results suggest that distinct maxillary beak phenotypes within each line could be selected to help reduce SFP damage and improve bird welfare.

Key words: morphometrics, injurious feather pecking, principal components analysis, radiography, laying hen

INTRODUCTION

Chickens use their beaks for important functions, including feeding, drinking, plumage maintenance, and establishing social order; however, beyond these functions, birds can develop abnormal beak-related behaviors such as severe feather pecking (SFP). SFP is the pecking at, pulling, and removal of feathers by perpetrator birds, which can cause damage to the integument of the recipient 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 in pain, mortality, increased feed costs, and poor feed efficiency (McAdie and Keeling, 2000; Su et al., 2006). Beak treatment, the practice of shortening and blunting beak length via hot-blade or infrared methods, is used to pre-emptively limit SFP damage, but it does not address the cause of the behavior itself. Evidence that there are nociceptors in the beak and pain-related behaviors after the earliest beak treatment methods (Gentle et al., 1991; Gentle and McKeegan, 2007) has led to increasing demand to ban all forms of beak treatment and research investigating alternative methods of preventing and controlling SFP. The behavior has a low to moderate heritability (Su et al., 2005; Bennewitz et al., 2014; Lutz et al., 2016), so selective breeding against it is possible; however, incorporating behavioral data into breeding programs can be challenging. Traits related to SFP but easier to measure, such as...
Beak shape has been a marker of how genetic variability underlies the adaptation to environments and may partly lead to avian speciation (Grant and Grant, 1993; Lamichhaney et al., 2015; Tokita et al., 2016), so there is little doubt that beak shape is genetically determined. As a result, it is not surprising that the natural shape of the beak varies considerably within and between laying hen lines. Aspects of beak shape, such as the beak overhang (difference between maxillary and mandibular beak lengths), appear to be heritable (Knief et al., 2012; Icken et al., 2017). Hens with naturally shorter and blunter maxillary beaks showed improved feather cover and reduced mortality (Icken et al., 2017). This poses the question of whether poultry breeding companies can take advantage of this pre-existing variation in beak shape and genetically select hens whose beak shapes are less damaging during SFP behavior. To do so, the beak shape phenotypes must first be identified. Beak shape in poultry is mostly described using linear measurements such as length, width, and depth (Carruthers et al., 2012; Morrissey et al., 2016); however, more recent studies have described beak shape using geometric morphometrics (analysis of shape using landmark coordinates) (Dalton et al., 2017; Struthers et al., 2021).

Using 2-dimensional geometric morphometrics (image-based), Dalton et al. (2017) found significant variation in turkey beak shape and reported that sex and beak size strongly influenced shape. The beak shapes identified were similar to other avian species (Foster et al., 2008; Kulemeyer et al., 2009; Shao et al., 2016). Stange et al. (2018) used 3-dimensional geometric morphometrics to compare domesticated chicken breeds and wildfowl skull morphologies. The domesticated breeds occupied a much larger area of shape space and showed much higher variation in skull shape. Struthers et al. (2021) developed the novel capability of using radiography to apply geometric morphometrics in live, non-sedated laying hens. They identified premaxillary and dentary bone phenotypes within 2 White Leghorn pure layer lines. They found that the shape and size of the premaxillary bone differed significantly between the 2 genetic lines. This suggests that distinct bone, and possibly beak, shape phenotypes could be selected to help reduce the damage inflicted by SFP.

The current study used photographs of 2 pure White Leghorn laying hen lines obtained from data previously described (Struthers et al., 2021) to achieve the following objectives: 1) generate maxillary beak morphometric data, 2) characterize the within phenotypic variation in maxillary beak shape between these 2 populations, 3) examine the genetic and phenotypic relationship between the shape of the maxillary beak and the underlying premaxillary bone, and 4) examine the genetic and phenotypic relationship between maxillary beak shape, feather cover, and mortality during the laying cycle.

**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 (P61FA9171) and complied with UK regulations regarding the treatment of experimental animals (Home Office (UK), 2014).

**Animals and Housing**

Birds (n = 710) used for this study were from 2 pure line populations (Line A and B) of White Leghorn laying hens. Both Line A (n = 458) and Line B (n = 252) hens contributed to the 4-line breeding scheme of Lohmann Selected Leghorn (LSL) hybrid layers (Lohmann Breeders GmbH). All birds were 40 wk of age at the time of digital image and radiograph acquisition (Struthers et al., 2021). Hens from both populations were housed in conventional cages (n = 200) in family groups (full and/or half-siblings) with 5 hens per cage. All hens from both lines were housed in the same room within the same building and received the same feed. Hens were housed until 100 wk of age under challenge conditions (i.e., lower feed nutritional content [energy 10.9–11.1 MJ/kg; crude protein 15–15.5%]) set by the breeding company.

**Prior Radiograph Acquisition and Analysis**

To investigate the relationship between the shape of the beak and its underlying bones, left lateral head radiographs were previously collected from the same birds and analyzed using the methods described by Struthers et al. (2021). To summarize, the premaxillary bone (within the maxillary beak) in each radiograph was landmarked in ImageJ (v.1.53g; National Institutes of Health, Bethesda, MD) using 13 landmarks. The 2D landmark coordinates were imported into R (v.3.6.1) and then R-based scripting and data visualization were done using the integrated development environment RStudio (v.1.4.1717) (R Core Team, 2019). Multivariate shape analysis was done using the R package Geomorph (v.4.0.1) (Adams et al., 2021). Within Geomorph, Procrustes superimposition was used to standardize all the landmarks in each radiograph by superimposing, rescaling, and rotating them all to a common orientation. The covariation of the transformed landmarks was analyzed using principal components analysis and multivariate regression.

**Image Acquisition**

Immediately following radiograph acquisition (Struthers et al., 2021), each hen was placed laterally against a plastic container secured to the wall and a digital image was taken. A 0.8-cm diameter green dot sticker was placed on the container to be used as a size standard during analyses. Images were captured using a Nikon
COOLPIX B700 digital camera (Nikon Corp., Tokyo, Japan) mounted to an adjustable camera tripod. After verification of a useable image (e.g., no blurriness detected from the camera’s LCD viewscreen), the hens were placed into a transport trolley and returned to their home cages. A total of 710 images (1 per hen) were collected.

**Image Analysis**

**Post Hoc Formatting** During image acquisition, the bird’s mouth was not open, causing the mandibular beak to be partially obscured by the maxillary beak in most of the images. As a result, it could not be landmarked reliably; therefore, it was decided to forego landmarking and analysis of the mandibular beak. For the maxillary beak, left lateral images (JPEG format) were excluded from landmarking if, despite checking during image acquisition, the image was blurry or if the landmarks could not be properly placed on their locations (e.g., the comb was obstructing landmark placement). This resulted in 637 lateral images being available for maxillary beak landmarking.

**Placement of Landmark Coordinates** The images were landmarked in ImageJ using the Multi-Point Tool. The anatomical terms of the location used to describe the placement of the landmarks and the shape of the maxillary beak are provided in Figure 1a. Twenty landmarks and semilandmarks (LMs) were chosen for the maxillary beak (Figure 1b). Five discrete LMs, that is, those with the same relative position (homology) between specimens, were placed first (Zelditch et al., 2012). LMs 6 to 20 consisted of sliding semilandmarks (Gunz and Mitteroecker, 2013). These LMs slid along the curves of the beak to minimize shape differences (Bookstein, 1991; Gunz and Mitteroecker, 2013). The 2D landmark coordinates from ImageJ were visualized using the integrated development environment RStudio (v.1.4.1717) (R Core Team, 2019).

**Geometric Morphometric Analysis** Multivariate shape analysis was done using the Geomorph package in R (v.4.0.1) (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 617 lateral maxillary beak images. Using Procrustes superimposition, the 2D landmark coordinates for all the images were standardized such that landmarks were superimposed across a common origin (centroid), rescaled, and rotated around the origin 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 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 (eigenvalues). The PCs with the largest eigenvalues (e.g., PC1 and PC2) were then plotted to establish the shape space of the birds.

The Procrustes superimposition also created a consensus beak shape by identifying the centroid (center point) of each photo’s landmarks and semilandmarks. The centroid size was then calculated as the square root of the sum of squared distances of landmarks and semilandmarks to the centroid (Zelditch et al., 2012). In the present study, centroid size served as a proxy for relative maxillary beak size independent of shape.

**Feather Cover**

All hens from both lines were individually feather scored at 40, 70, and 100 wk of age. At 40 and 70 wk, feather scoring was done by the same 2 trained observers. At 100 wk, due to COVID-19 restrictions, only one of the trained observers feather scored all the hens. The back, breast, wings, and tail were assessed (but not individually scored) and then an overall whole body feather

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**Figure 1.** (a) Schematic demonstrating the anatomical terms of the location used to describe maxillary beak shape. The terms are in relation to the white shaded area. (b) The discrete landmarks (LMs 1–5) and semilandmarks (LMs 6–20) used for the analysis of maxillary beak shape from left lateral images. LM 1, tip of maxillary beak; LM 2, rostral end of the nares; LM 3, caudal end of the nares; LM 4, junction between beak and comb/skin; LM 5, corner of mouth.
cover was scored on a scale of 1 to 9, with 1 indicating no feather cover and 9 indicating full, intact plumage (scoring scale provided by Lohmann Breeders). It was assumed that a hen’s beak shape would not influence its feather cover; therefore, a mean score per cage was calculated to determine the correlation between beak shape and feather cover.

**Mortality**

Birds were monitored daily for mortality and morbidity throughout the 100-wk production cycle by the breeding company farm staff. All mortalities were classified as either cannibalism-related or noncannibalism related. Specific causes of mortality could not be identified unless they were obvious (e.g., cannibalism) as birds were not sent for postmortem analysis.

**Statistical Analyses**

**Geometric Morphometric Analysis** A multivariate regression of all the Procrustes shape coordinates vs. log centroid size was performed to test for allometry (the relationship between size and shape). To determine if beak shape variation and allometry were unique between the 2 lines, a Procrustes ANOVA was performed. Data were analyzed using the procD.lm function within Geomorph (Adams et al., 2021). Significance was evaluated with a residual randomization permutation procedure with 1,000 iterations. Differences were considered significant when $P \leq 0.05$.

**Feather Cover and Mortality** Data were analyzed as a 1-way ANOVA in a completely randomized design with bird ($n = 710$) as the replicate unit for feather cover and cage ($n = 200$) for mortality. Data were analyzed using the R stats package (v.4.1.0) (R Core Team, 2019). Mortality percentage data were checked for normality and log transformed (data log + 1). Differences were considered significant when $P \leq 0.05$ and a trend was noted when $0.05 < P \leq 0.10$.

**Heritability and Genetic Correlations** To estimate heritabilities and genetic and phenotypic correlations between maxillary and premaxillary bone traits (PC scores), a bivariate model was fitted for each line in ASReml (v.4.1.1068; VSN International):

$$y = X\beta + Zu + e$$

where $y$ is a vector containing the phenotypic values for each trait for all birds, $\beta$ is a vector of fixed effects, and $u$ is a vector of random genetic effects. $X$ and $Z$ are the known design matrices for $\beta$ and $u$, respectively, and $e$ is a vector of residual errors. Narrow-sense heritability ($h^2$) was calculated as follows:

$$h^2 = \frac{\sigma^2a}{\sigma^2p}$$

where $\sigma^2a$ is the additive genetic variance and $\sigma^2p$ is the phenotypic variance.

**Spearman Correlations** Using the combined data from both lines A and B, Spearman correlations using the cor.test function in the R stats package (v.4.1.0) (R Core Team, 2019) were performed to test the relationships between maxillary beak shape and size, mortality (total and cannibalism-related), and feather cover. The degrees of correlation were defined as: 0.00 to 0.20 indicating a negligible correlation; 0.21 to 0.40 indicating a weak correlation; 0.41 to 0.60 indicating a moderate correlation; 0.61 to 0.80 indicating a strong correlation; and 0.81 to 1.00 indicating a very strong correlation.

**RESULTS**

**Maxillary Beak**

For the maxillary beak, the first 3 PCs were chosen as each accounted for more than 10% of the proportion of variance (Table 1). Together, the 3 PCs explained 83% of the total shape variation in the 2 populations of pure line laying hens. PC1 accounted for 46% of the total variation and partially separated the 2 lines. Line A hens tended toward longer maxillary beak shapes, while Line B tended toward shorter (Figure 2). PC2 accounted for 26% of the maxillary beak shape variation and describes an inferior (PC2 min) vs. superior (PC2 max) shift of the landmarks that corresponds to the curvature of the beak tip (Figure 2). PC3 explained 11% of the shape variation and describes a widening (PC3 min) vs. narrowing (PC3 max) of the dorsal/ventral margins of the maxillary beak that corresponds to beak depth (Figure 3).

The multivariate regression revealed that the shape coordinates were significantly associated with beak size ($Z$-score = 7, $P < 0.01$) and that beak size accounted for 14% of the total shape variation. Based on the Procrustes ANOVA, maxillary beak shape differed significantly between the 2 lines ($Z$-score = 7, $P < 0.01$). Allometry also differed between the lines as the interaction between log centroid size and the line was significant ($P = 0.02$). Line A hens had larger log centroid sizes (mean = 7.11 ± 0.08) than Line B (mean = 6.85 ± 0.05). Because this interaction was significant, no attempt was made to explore allometry-corrected shape.

**Table 1.** Proportion of premaxillary beak shape variance explained by the first 10 principal components (accounting for 97% of the total variance).

<table>
<thead>
<tr>
<th>Principal component</th>
<th>Eigenvalue</th>
<th>Proportion of variance</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00149</td>
<td>0.46</td>
<td>0.039</td>
</tr>
<tr>
<td>2</td>
<td>0.00084</td>
<td>0.26</td>
<td>0.029</td>
</tr>
<tr>
<td>3</td>
<td>0.00034</td>
<td>0.11</td>
<td>0.018</td>
</tr>
<tr>
<td>4</td>
<td>0.00014</td>
<td>0.04</td>
<td>0.012</td>
</tr>
<tr>
<td>5</td>
<td>0.00011</td>
<td>0.03</td>
<td>0.011</td>
</tr>
<tr>
<td>6</td>
<td>0.00009</td>
<td>0.03</td>
<td>0.009</td>
</tr>
<tr>
<td>7</td>
<td>0.00005</td>
<td>0.01</td>
<td>0.007</td>
</tr>
<tr>
<td>8</td>
<td>0.00004</td>
<td>0.01</td>
<td>0.006</td>
</tr>
<tr>
<td>9</td>
<td>0.00002</td>
<td>0.01</td>
<td>0.005</td>
</tr>
<tr>
<td>10</td>
<td>0.00002</td>
<td>0.01</td>
<td>0.004</td>
</tr>
</tbody>
</table>

1Standard deviation of the principal components, calculated as the square root of the eigenvalues.
Throughout the production cycle, feather scores for all birds remained high (>7), indicating good feather cover. However, Line A hens had better scores at all 3 ages than Line B (Table 2).

**Mortality**

Mortality differed significantly between the 2 genetic lines throughout the 100 wk production cycle. Line A hens had less overall mortality and cannibalism-related mortality than Line B (Table 3). There was also a trend for Line A hens to have less noncannibalism-related mortality.

**Heritability and Genetic Correlations**

**Premaxillary Bone** Estimates of heritability for premaxillary bone shape traits were low to moderate (0.10−0.57) for both genetic lines (Table 4). A positive genetic correlation was found between premaxillary bone curvature (PC1 score) and premaxillary bone size.
Table 2. Mean feather cover scores (out of 9)1,2 between 2 pure line populations of White Leghorn laying hens at 40, 70, and 100 wk of age.

<table>
<thead>
<tr>
<th>Age (wk)</th>
<th>Line</th>
<th>A</th>
<th>B</th>
<th>P value</th>
<th>SEM3</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>A</td>
<td>9.0</td>
<td>8.8</td>
<td>&lt;0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>70</td>
<td>B</td>
<td>8.1</td>
<td>7.7</td>
<td>&lt;0.01</td>
<td>0.21</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>7.7</td>
<td>7.3</td>
<td>&lt;0.01</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Differences considered significant when P ≤ 0.05.
1Score of 1 = no feather cover and 9 = full, intact feather plumage.
2Unless obvious (e.g., cannibalism), specific causes of mortality could not be determined.
3Standard error of the mean.

Table 3. Total mortality and mortality by cause (as a percent of birds housed) between 2 pure line populations of White Leghorn laying hens over 100-wk production cycle1.

<table>
<thead>
<tr>
<th>Mortality type</th>
<th>Line</th>
<th>A</th>
<th>B</th>
<th>P value</th>
<th>SEM3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td>10.7</td>
<td>16.7</td>
<td>0.03</td>
<td>1.29</td>
</tr>
<tr>
<td>Noncannibalism related2</td>
<td></td>
<td>10.3</td>
<td>14.3</td>
<td>0.07</td>
<td>1.11</td>
</tr>
<tr>
<td>Cannibalism-related</td>
<td></td>
<td>0.4</td>
<td>2.4</td>
<td>0.02</td>
<td>0.45</td>
</tr>
</tbody>
</table>

Differences considered significant when P ≤ 0.05 and a trend noted when 0.05 < P ≤ 0.10.
1Hens were housed under challenge conditions (lower feed nutritional content).
2Unless obvious (e.g., cannibalism), specific causes of mortality could not be determined.
3Standard error of the mean.

for both lines. Both lines also had a positive phenotypic correlation between premaxillary bone curvature (PC1 score) and bone size. In Line A, significant positive phenotypic correlation estimates were found between premaxillary bone curvature (PC1 score) and bone depth (PC3 score), premaxillary bone curvature (PC1 score) and bone size, and premaxillary bone depth (PC3 score) and bone size. In both lines, negative phenotypic correlation estimates were found between premaxillary bone curvature (PC1 score) and bone length (PC2 score), as well as premaxillary bone length (PC2 score) and bone size.

Maxillary Beak
Heritability estimates for maxillary beak shape traits were moderate to high (0.19–0.74) for Line A hens and low to moderate (0.07–0.55) for Line B (Table 4). In both lines, a negative phenotypic correlation estimate was found between beak length (PC1 score) and beak depth (PC3 score). Line B hens also had a negative phenotypic correlation between beak curvature (PC2 score) and beak depth (PC3 score).

Premaxillary Bone Vs. Maxillary Beak
In Line A, maxillary beak depth (PC3 score) showed a negative genetic correlation with premaxillary bone size but positive phenotypic correlations with premaxillary bone curvature (PC1 score) and premaxillary bone depth (PC3 score) (Table 4). In both lines, premaxillary bone length (PC2 score) was negatively phenotypically correlated with maxillary beak curvature (PC2 score).

Due to low estimates of heritability for maxillary beak shape traits in Line B, the estimates of genetic correlation between maxillary beak length (PC1 score), maxillary beak curvature (PC2 score), maxillary beak width (PC3 score), and premaxillary bone curvature (PC1 score) could not be done. In addition, for both lines, heritability and correlations were not estimable for maxillary beak size due to low sample size and phenotypic variance within the lines.

Spearman Correlations
No significant correlations were found between the maxillary beak shape traits (PC scores) and mortality

Table 4. Estimates of heritability (diagonal), genetic correlations (below the diagonal), and phenotypic correlations (above the diagonal), with standard errors, between premaxillary bone1 and maxillary beak2 shape and size traits (principal component (PC) scores).

<table>
<thead>
<tr>
<th>Line</th>
<th>Premaxillary bone</th>
<th>Maxillary beak</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PC1</td>
<td>PC2</td>
</tr>
<tr>
<td>A Premaxillary bone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC1</td>
<td>0.41 ± 0.14</td>
<td>−0.26 ± 0.05</td>
</tr>
<tr>
<td>PC2</td>
<td>−0.17 ± 0.23</td>
<td>0.53 ± 0.15</td>
</tr>
<tr>
<td>PC3</td>
<td>0.05 ± 0.43</td>
<td>−0.47 ± 0.45</td>
</tr>
<tr>
<td>Size</td>
<td>0.61 ± 0.15</td>
<td>0.07 ± 0.25</td>
</tr>
<tr>
<td>Maxillary beak</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC1</td>
<td>0.18 ± 0.37</td>
<td>0.30 ± 0.32</td>
</tr>
<tr>
<td>PC2</td>
<td>0.12 ± 0.24</td>
<td>−0.19 ± 0.22</td>
</tr>
<tr>
<td>PC3</td>
<td>0.13 ± 0.21</td>
<td>−0.08 ± 0.20</td>
</tr>
<tr>
<td>Size</td>
<td>n.e.</td>
<td>n.e.</td>
</tr>
<tr>
<td>B Premaxillary bone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC1</td>
<td>0.10 ± 0.12</td>
<td>−0.22 ± 0.05</td>
</tr>
<tr>
<td>PC2</td>
<td>−0.11 ± 0.47</td>
<td>0.28 ± 0.14</td>
</tr>
<tr>
<td>PC3</td>
<td>−0.06 ± 0.35</td>
<td>−0.11 ± 0.31</td>
</tr>
<tr>
<td>Size</td>
<td>0.70 ± 0.20</td>
<td>−0.17 ± 0.35</td>
</tr>
<tr>
<td>Maxillary beak</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC1</td>
<td>n.e.</td>
<td>0.38 ± 1.13</td>
</tr>
<tr>
<td>PC2</td>
<td>0.80 ± 0.58</td>
<td>−0.33 ± 0.37</td>
</tr>
<tr>
<td>PC3</td>
<td>0.28 ± 1.41</td>
<td>−0.42 ± 1.65</td>
</tr>
<tr>
<td>Size</td>
<td>n.e.</td>
<td>n.e.</td>
</tr>
</tbody>
</table>

Values (± standard error) in bold are considered significant (P ≤ 0.05).
1Premaxillary bone shape traits: PC1 = bone tip curvature; PC2 = bone length; PC3 = ventral bone margin depth (Struthers et al., 2021).
2Maxillary beak shape traits: PC1 = beak length; PC2 = beak tip curvature; PC3 = beak depth.
BEAK SHAPE VARIATION IN PURE LINE LAYING HENS

Table 5. Spearman correlation coefficients1 of maxillary beak shape and size characteristics (principal component (PC) scores)2,3 with mortality and feather cover.

<table>
<thead>
<tr>
<th>Maxillary beak</th>
<th>Mortality</th>
<th>Feather cover</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Cannibalism</td>
</tr>
<tr>
<td>PC1</td>
<td>−0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>PC2</td>
<td>−0.05</td>
<td>0.03</td>
</tr>
<tr>
<td>PC3</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>Size</td>
<td>−0.05</td>
<td>0.06</td>
</tr>
</tbody>
</table>

1Correlation degree (r); 0.00−0.20 = negligible; 0.21−0.40 = weak; 0.41−0.60 = moderate; 0.61−0.80 = strong; 0.81−1.00 = very strong.
2Using combined data from both lines A and B.
3Maxillary beak shape traits: PC1 = beak length; PC2 = beak tip curvature; PC3 = beak depth.

Values in bold are considered significant (P ≤ 0.05).

Avian beaks exhibit considerable variation in shape and size; however, the structures that form them follow similar patterns (Lucas and Stettenheim, 1972). All beaks comprise 2 bony projections or jaws, the maxillary and mandibular beak. The internal bone is covered by a layer of keratin known as the rhamphotheca, which thickens considerably as it approaches the beak tip (Lunam, 2005). This study aimed to characterize the maxillary beak shape of pure line laying hens using multivariate shape analysis. In laying hens, beak shape has previously been described using linear measurements such as length and width (Carruthers et al., 2012; Morrissey et al., 2016); however, these measurements do not reflect true shape differences.

The shapes of the maxillary beak described by the main axes of variation in the present study range from long and narrow with a more pointed beak tip to short and wide with a more curved beak tip. These shape patterns are similar to those found in commercial turkeys, tits, corvids, and finches (Foster et al., 2008; Kulemeyer et al., 2009; Shao et al., 2016; Dalton et al., 2017). The partial separation of the 2 lines across the axis of PC1 suggests that distinct maxillary beak phenotypes within each genetic line could be selected for (Figure 4). Incorporating multivariate shape data into selection indices could help breeding companies select hens whose beak shapes are less apt to cause damage when they engage in SFP behavior.

The beak shapes described in the present study are similar to the underlying premaxillary bone phenotypes that Struthers et al. (2021) identified. This is expected, given that there is a moderate correlation between the shape of the premaxillary bone and the maxillary beak in the present study. However, beak shape does not appear to simply reflect the underlying bone (Badikova and Dzerzhynsky, 2015; Urano et al., 2018). This is further supported by the existence of keratinous beak disorders such as cross-beaks in birds, including chickens, which only appear to affect the rhamphotheca and not the bone (Handel et al., 2010; van Hemert et al., 2012; Joller et al., 2018). In chickens, the premaxillary bone and the maxillary beak are similar in length and curvature, moving rostrally (toward the tip) until a certain point, after which they differ significantly (Urano et al., 2018). The reasons for this difference between the maxillary beak and the premaxillary bone are not yet fully understood. Urano et al. (2019) found that the portion of the rhamphotheca that covers the maxillary beak (also known as the rhinotheca) was comprised of 3 layers (outer, intermediate, and inner). The authors proposed that the outer and inner layers play a role in covering the dorsal and palatal surfaces of the rhinotheca, whereas the intermediate layer plays a role in the extension and thickening of the rhinotheca away from the premaxillary bone. This function of the intermediate layer may explain the differences in curvature and length between the maxillary beak and premaxillary bone (Urano et al., 2018, 2019).

In the present study, maxillary beak size accounted for less than 15% of the maxillary beak shape variation, similar to what has been reported in domestic turkeys (Dalton et al., 2017). Furthermore, size accounted for less shape variation in the maxillary beak than in the underlying premaxillary bone (Struthers et al., 2021). This suggests that other external factors influence beak shape, independent of size. Genetic variation that affects craniofacial morphogenesis are obvious candidates, as are those associated with beak shape differences in other avian populations (Abzhanov et al., 2004; Lamichhaney et al., 2015; Boer et al., 2021). In red junglefowl, their beaks frequently make contact with hard substances in the environment as part of their search for food, allowing for natural abrasion of the beak (Banks, 1956). Laying hens with intact beaks have been found to have minor changes in beak length, suggesting that, like red junglefowl, they experience some degree of natural beak abrasion within their environment (Gabrush, 2011). Alteration of the beak shape by beak blunting (use of abrasive materials or enrichment devices to naturally blunt and shorten beak length) has also been studied. However, while some studies report beak length reductions with the use of blunting devices (Grim et al., 2021; Baker et al., 2022), others do not (Morrissey et al., 2016; Iqbal et al., 2020; Struthers et al., 2022). More importantly, none of these studies found that beak-blunting devices helped reduce feather damage or mortality.

The heritability estimates for bone and beak shape traits in both genetic lines were similar to what was reported in Lohmann LSL pure lines by Icken et al. (2017). This shows that the potential exists to reduce feather pecking damage by selection of specific beak shape phenotypes. The heritability and correlation estimates in the present study varied between the 2 genetic lines; however, both lines showed strong, positive genetic and phenotypic correlations between premaxillary bone

(total and cannibalism-related). Significant correlations were found between maxillary beak length (PC1 score), maxillary beak size, and mean feather cover at 40, 70, and 100 wk of age (Table 5); however, these correlations were weak to negligible.

DISCUSSION

(continued)
curvature (PC1) and bone size. This is not surprising given that premaxillary bone size accounts for nearly half of the total premaxillary bone shape variation (Struthers et al., 2021). Pronounced allometry is a common theme between the genetic lines and appears to be driving the premaxillary bone curvature phenotype. Given the multifactorial nature of SFP, the results of the present study are not meant to act as a replacement for the current management and genetic practices already used to reduce the incidence and severity of the behavior (e.g., family selection for feather cover and livability). Rather, they help contribute to the array of resources already in place and provide yet another “tool” for helping improve the welfare of intact-beak laying hens worldwide. Further research could focus on determining the genetic correlations between the bone and beak shape traits characterized in the present study and other behavioral and production traits.

Beak treatment, regardless of method, has been shown to alter maxillary beak and premaxillary bone shape (Gentle et al., 1997; McKeegan and Philbey, 2012; Struthers et al., 2019b), resulting in a reduction in feather damage (Marchant-Forde et al., 2008; Riber and Hinrichsen, 2017; Struthers et al., 2019a). It is not yet fully understood if this can be directly related to naturally occurring beak shapes. Feather cover is an important component of hen health and welfare. Poor feather cover increases the risk of further pecking which can trigger cannibalism and can cause heat loss, increased feed consumption, and poor feed efficiency (McAdie and Keeling, 2000; Su et al., 2006). It is hypothesized that hens with blunt maxillary beaks are less able to grasp and pull feathers and body tissue. Icken et al. (2017) found that hens with naturally shorter maxillary beaks tended to have better feather cover. However, caution should be used when comparing their results to the present study as Icken et al. (2017) measured beak overhang length (i.e., the difference between the maxillary and mandibular beak lengths), a univariate or linear measurement. In contrast, the present study used multivariate measures of shape. The present study suggests a relationship between feather cover and beak shape. The correlation between maxillary beak length (PC1 score) and mean feather cover suggests that as PC1 score increases (i.e., move toward the right-hand side of the PC1 axis), so does feather cover. The fact that Line A hens, whose beaks were larger and longer (i.e., beak shapes skewed toward the right of the PC1 axis) had better feather cover compared to Line B hens supports this observation (Figure 3).

Icken et al. (2017) also reported that hens with naturally shorter maxillary beaks tended to have lower mortality. The genetic correlations between mortality and beak overhang length for the different lines were mostly positive, indicating that selection for blunter beaks could reduce mortality (Icken et al., 2017). Although differences in feather cover and mortality were observed between the lines in the present study, the cause of these differences is unclear. What was not measured was whether 1 line engaged in more SFP behavior than the other, causing more feather loss and mortality. The pecking behavior of hens with different beak shapes characterized using geometric morphometrics has never been quantified. It is possible that nonbehavioral factors contributed to the differences in feather cover and mortality; however, cannibalism-related mortality was 6 times higher in Line B. This strongly suggests that SFP or some other form of injurious pecking (e.g., vent

![Figure 4. The maxillary beak shape variation at the minimum, mean, and maximum values of PC1, PC2, and PC3, representing beak length, curvature, and depth, respectively.](image-url)
beak-related behaviors such as feeding and preening. The present study is one in a series characterizing beak-related behaviors such as feeding and preening. The future research could control for SFP behavior by recording occurrences of feather pecking within a group and choosing the birds that perform the highest levels of feather pecking as test subjects, for example.

The results demonstrate that specific maxillary beak phenotypes could be selected for within genetic lines; however, to confidently incorporate multivariate shape data into selection indices, research investigating the physical damage these beak shapes can cause is needed. Future research could also investigate if these distinct beak phenotypes influence other beak-related behaviors such as feeding and preening. The present study is one in a series characterizing the shape of the beak and its underlying bones in laying hens. Subsequent studies will use this shape data to associate certain phenotypes with feather pecking-related damage.

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DISCLOSURES

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