

Use of a gel documentation system to measure feather growth bars

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ABSTRACT. Feather growth bar widths have become a standard index of nutritional condition in ornithological research. Traditional techniques of measuring these bars are difficult and slow because the bars are often hard to see. We present a fast and repeatable method of visualizing and measuring growth bars using a gel documentation system from Alpha-Innotech Corporation. This system takes digital photos of objects in a low light chamber. These photos can then be enhanced and measured on a computer. We show that this system can be used to quickly obtain repeatable measurements of growth bars that are highly correlated with those obtained using traditional methods. The high repeatability and low inter-observer variability of our results also validates more traditional methods of ptilochronology.

SINOPSIS. **Uso de un sistema de documentación de gelatina para medir las barras de crecimiento en plumas**

La amplitud de las barras de crecimiento en las plumas se han convertido en el índice básico de la condición nutricional en estudios de aves. Las técnicas tradicionales de medir estas barras son difíciles y lentas porque las barras a menudo son difíciles de ver. Presentamos un método rápido y repetible de visualizar y medir la barras de crecimiento usando un sistema de documentación de gelatinas de la Corporación Alpha-Innotech. Este sistema toma fotos digitales de objetos en una cámara con luz baja. Estas fotos pueden ser modificadas y medidas en una computadora. Mostramos que este sistema puede ser usado para obtener rápidamente medidas repetidas de barras de crecimiento que son altamente correlacionadas con las obtenidas por los métodos tradicionales. Tanto la alta capacidad de repetir las medidas como la baja variabilidad de resultados entre observadores validan este método como útil entre otros más tradicionales en la ptilocronología.

Key words: digital imaging, House Finch, nutritional condition, ptilochronology

Ptilochronology, or the use of feather growth bars as an index of nutritional condition, has been used extensively since Grubb (1989) introduced the concept. Feathers have a series of light and dark bands oriented obliquely to the rachis. Each light and dark band taken together (one growth bar) represents 24 h of growth (Riddle 1908; Michener and Michener 1938), and evidence suggests a direct relationship between width of these bars and nutritional condition (Grubb 1989, 1991, 1995; Hill and Montgomerie 1994; Jenkins et al. 2001). Since 1989, researchers have used ptilochronology to study relationships between nutritional condition and territory quality (Grubb et al. 1998; Stratford and Stouffer 2001), plumage color (Hill and Montgomerie 1994), and reproductive effort, social dominance, and territory size (reviewed in Grubb 1995). Ptilochronology is

thus a powerful tool for research in avian ecology.

Two methods have been used to measure growth bars. In the first method (Grubb 1989), feathers are taped to index cards. Growth bars are marked on the index card by poking through the feather with a small mounting pin in the center of the dark bar along the rachis of the feather. The distances between adjoining holes on the card are measured, and the mean width is calculated by dividing the aggregate width by the number of growth bars. In the second method, growth bars are measured directly using calipers, and their mean width is calculated as above (Møller 1994).

Problems with the ptilochronology technique can arise because of human error and the different abilities of people to distinguish growth bars (Murphy and King 1991). Often they are visible only after extensive adjustment of feather position, and even then may be blurry and indistinct. Furthermore, measurement of growth bars is time-consuming. The difficulty of these

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methods has prevented them from being more widely applied. Until now, however, they have been the only options available to those interested in using ptilochronology. Here we describe a novel technique for rapid visualization and measurement of growth bars using a gel documentation system (Alpha-Innotech 2002). These systems take digital photos of objects under low light conditions and are used primarily to quantify gels and bacterial cultures. We compare results obtained using this system with those obtained using the first method. The use of the gel documentation system increases the speed and ease of growth bar measurement and should facilitate the use of ptilochronology in future studies.

METHODS

From 1998–2001, we removed the outermost retriex from 23 juvenile House Finches (*Carpodacus mexicanus*) captured in feeder traps in Lee County, Alabama. In 2002, we measured the growth bars on these feathers using both Hill and Montgomerie's (1994) modification of Grubb's (1989) technique and a gel documentation system at Auburn University. To test their repeatability, two observers (M.D.S. and M.L.B.) measured each feather three times using both methods.

Traditional method. We first taped the quill of each feather to a piece of black construction paper, to increase the visibility of the bars. We pushed a size 0 insect pin through the middle of the most proximate, clearly visible dark band, and through the middle of the dark band nine growth bars distal from it. If nine bars were not visible, we counted the maximum number possible. We then removed the feather and measured the distance between the two holes in the construction paper using a ruler. To obtain average bar width, we divided the total distance by the number of growth bars counted.

Gel documentation system method. The gel documentation system (Alpha-Innotech, San Leandro, CA) consists of a Mutilmage[™] II light cabinet with white lights on the right and left uppermost side, a high-performance CCD camera with a 12.5 × 75 mm zoom lens, a close-up +2 diopter lens with interference filter, and a computer with AlphaImager[™] for Windows software. Magnification and f-stop set-

tings can be adjusted on the camera itself. The user can view objects in the light cabinet on the computer monitor and adjust the levels of black and white in the image, as well as the gamma (brightness) setting to maximize both contrast and resolution. All images appear in gray-scale. A digital image can be captured using the "Freeze" option. This image can then be further magnified using the Alphaimager software. The "Ruler" function allows the user to measure the distance between two points on the screen after calibration to a known scale (such as a ruler) within the image. It is necessary to calibrate the digital ruler for each image if any changes in magnification are made.

To visualize growth bars, we placed the feather on black construction paper in the light cabinet. We positioned a ruler near the feather for use as a scale marker. Growth bars were most clearly visible when one of the two cabinet lights was shaded using a piece of cardboard or construction paper. We made further adjustments using the light aperture and zoom feature on the camera and the "Black," "White" and "Gamma" settings on the computer. Each feather required slightly different settings. For example, some growth bars were visible when the right-hand light was shaded, while for others the opposite was true. The ideal f-stop and zoom settings also differed between feathers. We used between 12.5 and 25-mm zoom; thus, it was necessary to calibrate the digital ruler for each picture.

We calibrated the digital ruler using the 1-cm mark of our scale marker in each image. We then placed one end of the digital ruler in the center of the most proximate, clearly visible dark band, and measured to the center of the band nine full growth bars distal to it. As in the traditional method, we counted as many growth bars as possible and obtained average bar width by dividing the total distance by the number of growth bars measured.

Analyses. All analyses were performed using the SPSS statistical package for the Macintosh (SPSS 2000). Repeatability was measured as the intraclass correlation coefficient (Lessels and Boag 1987). We used one-way ANOVA with measurements nested within feathers as our replicates to test for differences in growth bar width measurements by the two observers. We found no significant differences between observers for either method, so we

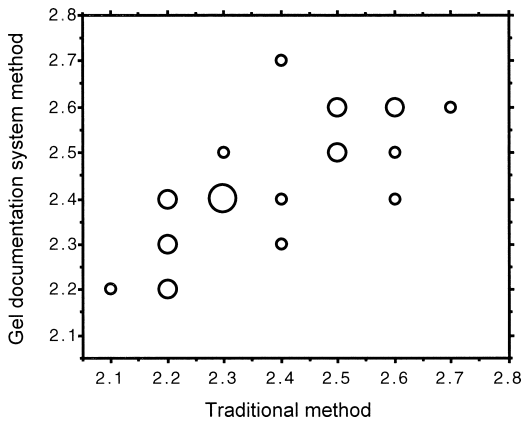


Fig. 1. Measurements (mm) of growth bar widths of House Finch feathers ($N = 23$) taken using Hill and Montgomerie's (1994) modification of Grubb's (1989) method in relation to those taken using a gel documentation system (Alpha-Innotech 2002). The size of the circle indicates the number of overlapping points; the smallest circles represent one point, the next largest represent two points, and the largest represent three points.

pooled the measurements for each method. We then used one-way ANOVA on these pooled results, with measurements nested within feathers as our replicates, to test for differences between methods. We used Pearson's correlation to determine the relationship between the measurements obtained using the two methods.

RESULTS

The repeatability of both methods was high and significant (traditional, $R = 0.79$, $F_{23,115} = 5.30$, $P < 0.0001$; gel documentation system, $R = 0.81$, $F_{23,115} = 4.70$, $P < 0.0001$). The measurements obtained using both techniques were not significantly different between observers (ANOVA, traditional, $F_{1,45} = 2.34$, $P = 0.13$; gel documentation system, $F_{1,45} = 1.87$, $P = 0.18$). The pooled results of the two techniques were not significantly different (ANOVA, $F_{1,45} = 2.68$, $P = 0.11$). Pearson's correlation revealed a highly significant relationship between the pooled results for the two methods ($r^2 = 0.47$, $N = 23$, $P < 0.001$; Fig. 1).

DISCUSSION

The reliability of growth bar measurements has been questioned (Murphy and King 1991).

We performed repeated measurements of growth bars with two observers for both methods, and found no between-observer differences and high repeatability. Our correlational data show a strong similarity between the methods. Thus, both methods are reliable and produce similar results.

The gel documentation system has proven to be an excellent tool for ptilochronology. Growth bars are more easily visualized on a computer screen than in hand, and the control of the camera settings allows for increased contrast and resolution. Thus, it is easier to measure growth bars using this system. Taking a permanent digital photo enables the user to establish a database to follow individuals over time. This new method is also fast. Once we perfected the technique, we measured 23 feathers three times each in about four hours, which is less than half the time that it took using the traditional method. Finally, the gel documentation system minimizes feather damage by lowering the amount of manipulation and eliminating the use of mounting pins.

Although we only tested one species in this paper, preliminary results indicate that the method works equally well for Eastern Bluebirds (*Sialia sialis*), and we do not foresee any difficulties in working for other species. The gel documentation system is expensive (c. \$15,000 U.S.), but these systems are now standard equipment in all molecular biology labs and thus should be accessible to ornithologists at universities.

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