Do songbirds store calcium in their legs?

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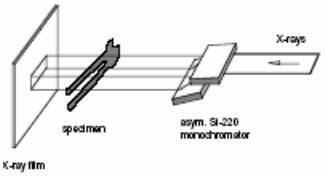
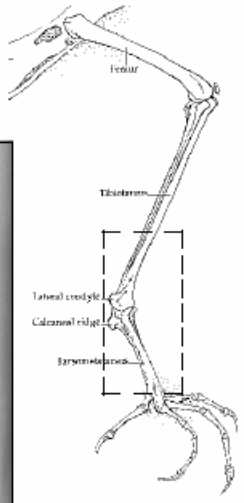
During egg laying, birds must mobilize large amounts of fat, protein and minerals, especially calcium, for the formation of their eggs. Of these three, calcium has been the least well studied. Observations on chickens and pigeons have suggested that the formation of large amounts of "medullary bone" in hollow spaces in the interiors of their leg bones could serve as a store of easily mobilized calcium to provide the large amounts of calcium required for egg shell formation [1,2]. There have apparently never been any studies of the development of medullary bone in laying passerine birds.

There are thought to be two basic strategies of resource use in birds preparing for reproduction: those that accumulate resources over a long period prior to reproduction, storing essential resources in somatic "bank accounts" for later reproduction. These so-called "capital" breeders can be contrasted with "income" breeders which do not store resources but lay eggs on resources that are ingested very shortly before egg-laying. Greater understanding of which of these strategies are pursued by songbirds have become of more than basic interest in recent years, as increasing evidence is accumulating to indicate that reproductive failures in some songbirds can be linked to decreases in calcium availability due in part to the effects of acid rain.



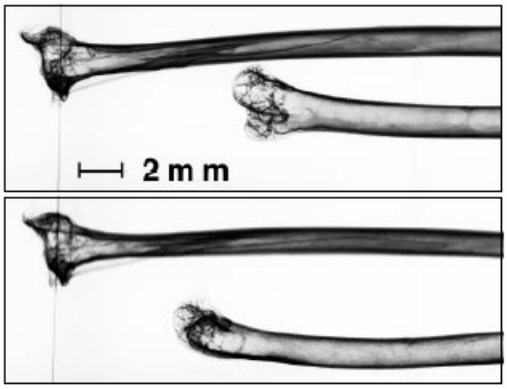
(Figure 2) Schematic view of the experimental setup for the radiography measurements.

For the past year, we have been studying the structure of the leg bones of songbirds to see whether these birds are best interpreted as income or capital breeders with regards to their calcium metabolism. In a series of experiments on legs from passerine birds we have generated x-ray images that indicate the potential for quantifying calcium stores in live birds without harming them. The leg bones of these birds are tiny (see Fig. 1, about 14 mm long and 1.5 mm in diameter), and contain about 60% minerals, 38% of which are calcium, other components are fat, collagen, and water. The challenge has been to visualize the interiors of the bones with sufficient acuity to measure the differential development of calcium deposits within them [3].



(Figure 1) Skeletal view showing the structural characteristics of bird leg bones. The inset shows leg bones from Tachycinets bicolor and the location of the radiograph.

The experiments were carried out at CHESS beamline D1 using X-rays at 12 keV (figure 2). We used an asymmetric-cut Si-220 monochromator to obtain a beam with a large cross section and high energy resolution ($\Delta E/E=10^{-4}$). Radiographs of the bone samples were stored on Kodak high resolution x-ray film. In order to allow for computerized data analysis the recorded information was digitized with a film scanner. The spatial resolution obtained through this process is 9 µm. The use of the synchrotron radiation (SR) at CHESS offers several advantages: The collinearity and the monochromatization of the SR eliminate distortions present in images made with conventional x-ray sources, providing higher spatial contrast and improved sensitivity for chemical elements. Most important here, the monochromaticity reduces the radiation dose to the specimen and the high intensity of the beam (approx. 10^9 photons/sec) allows for short exposure times (less than 0.4 sec-



(Figure 3, left) Lateral views of sections from femur and tibiotarsus of Molothrus ater captured during the breeding season: Female (top) and male (bottom).

onds), necessary for *in vivo* experiments with live birds.

In the first set of experiments, we measured the density and internal structure of the leg bones of tree swallows (*Tachycineta bicolor, Fig. 1*) before and after egg laying. We captured females in their nests before they laid any eggs and took detailed radiographs of their leg bones. Immediately after they completed their clutches, we captured the same females again, thus allowing us to compare the thickness, structure and density of their leg bones shortly before and immediately after laying. Clearly, capital breeders would be expected to exhibit larger amounts of calcium in the bones prior to laying and some evidence that these calcium stores were depleted by laying. In the tree swallows, we found no evidence of such an effect. The bones of females before and after laying were indistinguishable.

To test the robustness of this pattern, we turned to museum collections of the leg bones of brown-headed cowbirds (*Molothrus ater*). These birds are brood parasites, birds which trick other birds into raising their young for them. One of the hallmarks of this life-style is that females must lay extraordinary numbers of eggs to guarantee successful reproduction. So, while a female tree swallow may lay five to eight eggs in a season, a cowbird will often lay up to 50. Clearly, if there is any songbird that would have a use for somatic stores of calcium, the cowbird would appear to be it! Because we did not have access to females before and after laying, we compared the bones of female cowbirds collected during the reproductive season with those of females collected during the fall and with those of males, who clearly have no adaptive need for large calcium stores. These comparisons yielded the same conclusion as did the tree swallow studies: cowbird leg bones show no sign of thickening or structural changes associated with the breeding season, nor are female leg bones distinguishable from those of males (figure 3). Thus, cowbirds, too, appear to be income breeders for calcium.

We next turned to a sample of bones collected by our colleague, Dr. Jaap Graveland, at the Netherlands Institute of Ecology. Dr. Graveland's work on the effects of calcium limitation for the reproduction of great tits (*Parus major*) is a significant contribution to our understanding of the role of acid rain and calcium limitation in songbird reproduction [4]. In our experiments we compared the bones of tits that were collected or died of reproductive complications associated with attempting to breed in calcium-poor habitats with those from relatively calcium-rich habitats. Even though there were more differences among individual birds in these Dutch samples than we detected in the other two studies, there were no significant overall differences between the leg bones of adults breeding in calcium-poor and calcium-rich environment (figure 4 A-B).

In contrast to all these studies on adult birds, we did find substantial differences in the leg bones of great tit young that were reared in conditions of different calcium availability to their parents (figure 4 C-D). The specimen shown in figure 4C has a significantly thicker outer wall of the hollow bone and a more pronounced trabecular structure near the joint than specimen 4D, corresponding to a much higher content of calcium in the bones of the juvenile raised in a calcium-rich habitat. Our methods could thus detect differences in the calcium states of songbird leg-bones, but these differences are only apparent in those birds that have differing calcium availability during their initial development.

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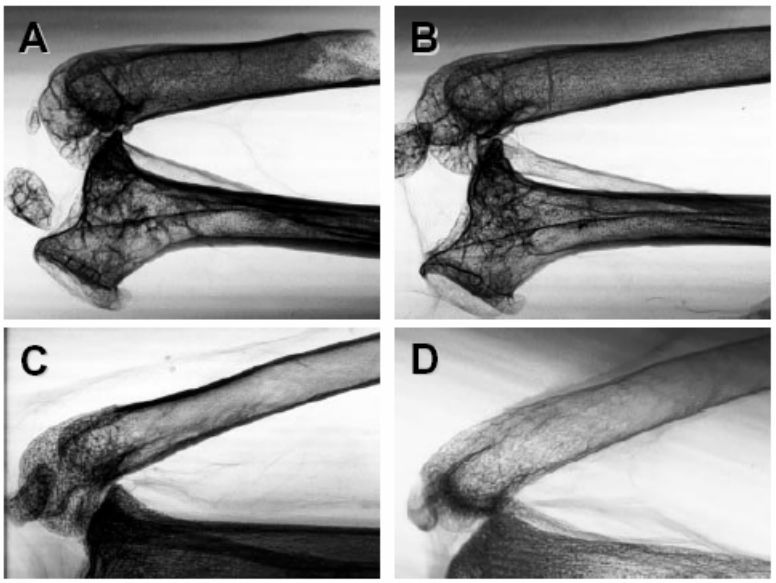

(Figure 4) Radiographs of the distal ends of femurs from great tits: A and B - adult females collected from calcium-rich and calcium-poor habitats. C and D - juveniles of same age raised in captivity simulating calcium-rich and calcium-poor habitats, respectively.

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In summary, the high resolution radiography made possible at CHESS has allowed us to determine with a fair degree of certainty that songbirds are not capital breeders. Rather, these birds must base the production of egg shells on their income of calcium over short time periods before and during the laying of a clutch. Thus, these species become very sensitive to any environmental changes, such as acid rain or chemical poisoning of soils. The decrease in availability of crucial minerals are likely to have direct and immediate impact on the reproduction of these birds.

1. C. D. Ankney, and D. M. Scott, "Changes in Nutrient reserves and diet of breeding brown-headed cowbirds," The Auk 97, 684-696, 1980.
2. A. B. Gilbert, "Calcium and reproductive function in the hen," Proc. Nutr. Sci. 42, 195-212, 1983.
3. Using a calibrated absorption standard we determined the sensitivity to bone minerals in our experiments to approx. 0.02 g/cm^3. The quantitative analysis of mineral contents in bones by radiography, especially the concentration of calcium, has previously been reported by other groups. See for instance: (a) E. Burattini, M Gambaccini, M. Marziani, O. Rimondi, P. L. Indovina, M. Pocek, G. Simonetti, M. Benassi, C. Tirelli, and R. Passariello, "X-ray mammography with synchrotron radiation," Rev. Sci. Instrum. 63, 638-641, 1992; (b) U. Bonse, F. Busch, O. Günnewig, F. Beckmann, R. Pahl, G. Delling, M. Hahn, and W. Graeff, "3D computed x-ray tomography of human cancellous bone at 8 µm spatial and 10^{-4} energy resolution," Bone and Mineral 25, 25-38, 1994.
4. J. Graveland, "The Quest for Calcium - Calcium limitation in the reproduction of forest passerines in relation to snail abundance and soil acidification," Ph.D. Thesis, University Groningen, Netherland, 1995.