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'Real time' observations of directional selection on murine tibiae and its correlated effects across the skeleton

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Introduction

Natural selection works on the variation between individuals to modify organismal morphology between species, as well as within a species[1]. The focus of this project is to investigate the extent relative tibia length increases in a line of CD1 mice when a directional selective pressure is applied. In addition, if tibia length changes significantly, will other limb bones (the femur, humerus and ulna) also change in length? If so, what are the relationships between these changes?

Figure 1: Variation within *Canis lupus familiaris*

There are three hypotheses for how the mouse skeleton will holistically respond to the selection:

(i) Overall larger mice are produced, hence larger bones

(ii) Serially homologous bones will show similar patterns of increase

(iii) Bones in the hind limb will respond better to selection than bones in the forelimb

Breeding Program and Data Collection

- 3 closed lines of 14 families of CD1 mice (2 *Selected* and 1 *Control* lines).
- At 8 weeks, measure body mass and tibia length within *Selected* line families, rank individuals based on absolute and relative tibia length.
- Select top-ranked male and female in each family to become breeders with members of other families (no sib-mating).
- In *Control* line, mice are selected to breed at random.
- µCT scan mice using Skyscan 1173 scanner (resolution of 44.7 µm).
- Using Amira 5.4.2 visualization software, the tibia, femur, humerus, ulna and skull are landmarked to determine bone length. These data were then analyzed.

Figure 2: A landmarked mouse tibia and femur

References

[1]Darwin, C. 1859. *On the origin of species by means of natural selection*. J. Murray, London.

Figure Sources:
Fig. 1: http://farfalle1.wordpress.com/elaborations/great-danes/
Fig. 6: http://www.bio.miami.edu/dana/106/106F05_4.html

Results

Relative tibia length of the F7 *Selected* generation shows a significant increase in length (**7.3%**, Fig. 3) when compared to the *Control* line. Overall, the tibia showed the largest mean increase in bone length. The next largest increase was in the femur (**6.4%**, Fig. 3), the ulna (**5.1**%, Fig. 3) then the humerus (**4.9**%, Fig. 3). The skull length increased minimally (**1.6%**, Fig. 3).

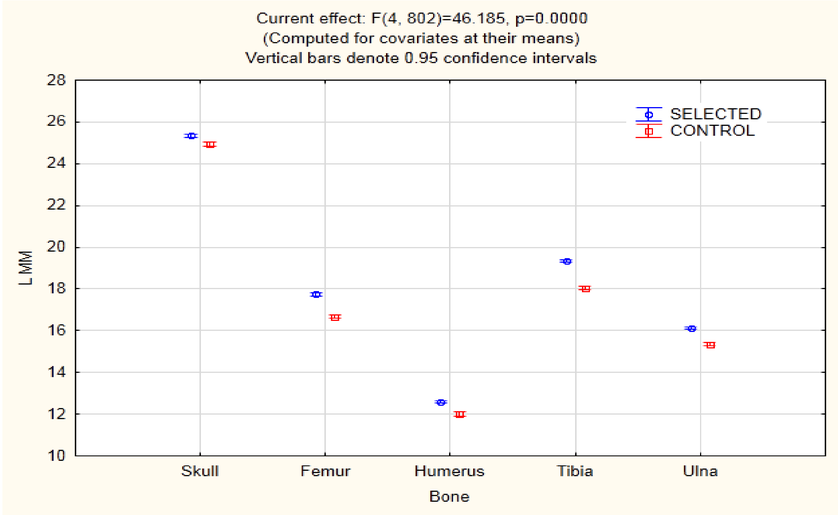

Figure 3: A comparison of various bones' mean length (mm) in the F7 *Selected* line and the *Control* line. Data are least-square means based on a GLM using the mouse line as a categorical factor and using age at measurement, cube-root of body mass and litter size as covariates.

The hind limb in this experiment was significantly more affected by the directional selection (**7.0%**, Fig. 4) than the forelimb (**5.2**%, Fig. 4), which supports hypothesis (iii).

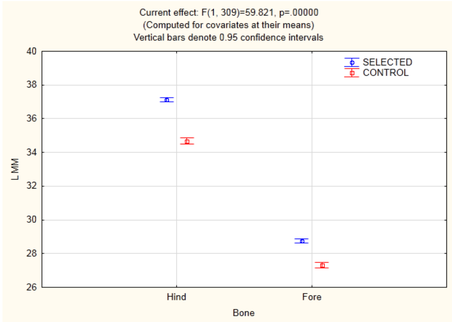

Figure 4: Mean overall limb length changes for the forelimb and hind limb of the F7 *Selected* as compared to the *Control* line

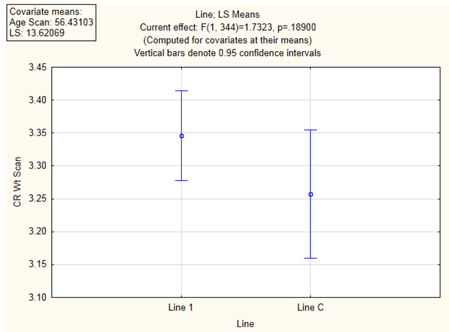

Figure 5: Mean body mass of F7 *Selected* mice as compared to the *Control* line

The mean body masses of the F7 *Selected* mice are slightly larger than the *Control* line, but did not reach statistical significance (p=0.189) (Fig.5).

Current and Future Analyses

As this project was only able to observe mice up to the F7 *Selected* generation, it would be beneficial to analyze later *Selected* generations of mice. This will provide further insight into rates of change in measured bones, and how or if these rates change with time.

In order to fully describe the rate of change of each bone's length, a full bone length analysis of each generation preceding F7 *Selected* should be carried out, so a comparison can be made across each generation.

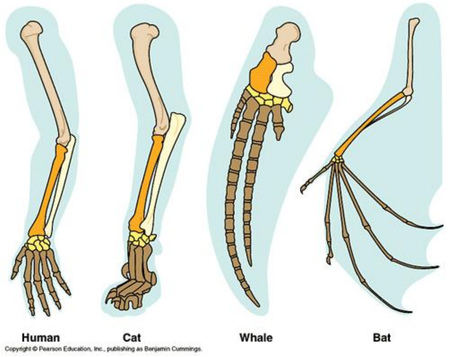

Figure 6: Diversity in homologous structures in mammals

Also, it might be beneficial to investigate changes in other aspects of bone morphology, such as any changes in shape or width of the bones observed .

Overall, further inquiry into the areas mentioned above will help show how variation relates to the generation of morphological diversity in vertebrates.

Significance

This project provides important insight into the heritability of bones across the skeleton and investigates the genetic basis of different aspects of limb-bone morphology. This research may also provide empirical support for a portion of evolutionary theory that has never been thoroughly tested before.

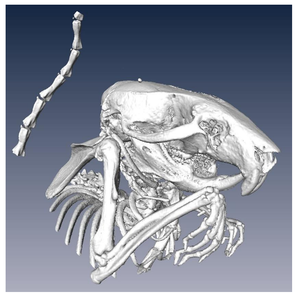

Figure 4: Forelimb and upper skeleton of a mouse

Acknowledgments

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