Title

Temperature-Dependent Variation in Flight Muscle Morphology Revealed by Micro-CT Imaging

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Big-Bee | USDA National Institute of Food and Agriculture, U.S. Department of Agriculture | UC Santa Barbara Cheadle Center for Biodiversity & Ecological Restoration

Introduction

Bees are important pollinators that support both natural ecosystems and agricultural production.[1] As climate change increases temperature variability, understanding how temperature affects bee development is important for predicting pollinator resilience.[2]

Flight muscles are especially important because they power flight, foraging, thermoregulation, and pollination. Recent work shows that increased temperature can disrupt bumble bee thoracic muscle performance, suggesting that flight-related muscle function may be sensitive to warming conditions.[3] However, because these muscles are internal, they are difficult to study using external measurements alone.

Here, we use high-resolution micro-CT imaging to visualize internal flight muscle anatomy in *Bombus impatiens* and examine whether developmental temperature is associated with differences in flight muscle morphology. Micro-CT imaging has been used to visualize small internal insect structures in three dimensions, making it useful for studying morphology that is difficult to measure externally.[4]

Methods

Temperature treatments

Two *Bombus impatiens* individuals were reared under different developmental temperature treatments: 10°C and 30°C.

Micro-CT imaging

Two specimens, one from each heat treatment, were scanned at 71 kV, 16 µA, 1.1 W, and 1.42 s exposure. Scans were acquired in continuous mode, reconstructed using dual high-quality settings without additional calibration, and saved as TIFF stacks.

Image processing

Micro-CT image stacks were cropped and prepared for analysis using FIJI.[5] The 10°C specimen image stack contained 993 images, while the 30°C specimen image stack contained 846 images.

Segmentation

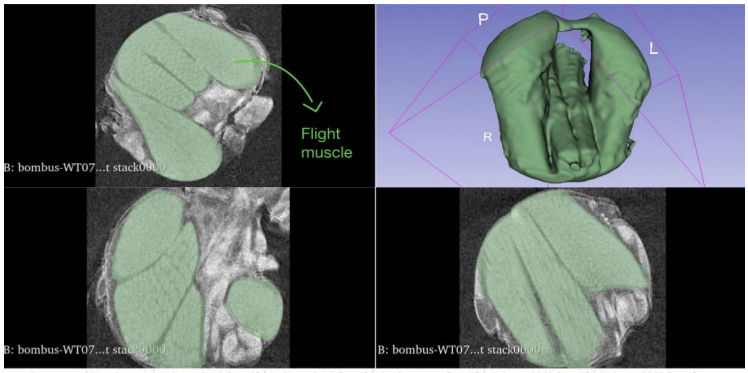
Flight muscle tissue within the mesosoma, or thorax, and the whole mesosoma were digitally isolated from surrounding anatomical structures using 3D Slicer version 5.10.0.[6]

Volume/SA analyses

Segmented flight muscle regions and whole mesosoma reconstructions were used to calculate relative volume and SA for comparison across temperature treatments.

Results

30°C Treatment: WT07
Flight muscle volume = 23.80 mm³



	Segment	Voxel count	Volume (LM) [mm3]	Volume (LM) [cm3]	Surface area [mm2]	Volume (CS) [mm3]	Volume (CS) [cm3]
1	Segment_1	2600952	23.7982	0.0237982	62.728	23.7944	0.0237944

Figure 1. Representative flight muscle segmentations from the 30°C and 10°C developmental temperature treatments. Green regions show digitally segmented flight muscle tissue in *Bombus impatiens* CT image stacks and 3D reconstructions. Preliminary flight muscle volume was 23.79 mm³ for the 30°C specimen and 19.91 mm³ for the 10°C specimen.

Measurement	30°C	10°C
Flight muscle volume [mm³]	23.7982	19.9164
Flight muscle SA [mm²]	62.728	60.1685
Mesosoma volume [mm³]	42.6966	95.6
Mesosoma SA [mm²]	101.7537	200.0086
Relative muscle volume [%]	55.74	20.83
Relative muscle SA [%]	61.65	30.08

Table 1. Scaled flight muscle and mesosoma measurements by temperature treatment.

Discussion

The 30°C reared specimen had a higher relative flight muscle volume than the 10°C reared specimen, with flight muscle occupying 56% of the mesosoma volume compared with 21%. Relative flight muscle surface area showed a similar pattern, representing 62% of mesosoma surface area in the 30°C specimen compared with 30% in the 10°C specimen.

Heat may affect flight muscles by altering development, metabolism, and tissue growth. Because bee flight muscles power wing movement and generate heat for thermoregulation, changes in relative muscle size or structure could influence flight performance and temperature tolerance. Overall, this project demonstrates that micro-CT imaging and 3D segmentation can be used to visualize and quantify internal bee flight muscle anatomy.

However, because these measurements are based on only two representative specimens, they are not yet sufficient to draw confident conclusions about temperature effects.

Figure 2. Study organism: *Bombus impatiens*, the common eastern bumble bee. Photo by scibadger via iNaturalist, CC BY-NC-ND.[7] Wings attach to the mesosoma, which contains the flight muscles analyzed in this study.

References

1. Katumo, D. M., Liang, H., Ochola, A. C., Lv, M., Wang, Q., & Yang, C. F. 2022. Pollinator diversity benefits natural and agricultural ecosystems, environmental health, and human welfare. *Plant Diversity*, 44(5), 429–435.
2. Gérard, M., Cariou, B., Henrion, M., Descamps, C., & Baird, E. 2022. Exposure to elevated temperature during development affects bumblebee foraging behavior. *Behavioral Ecology*, 33(4), 816–824.
3. Woodrow, C., Sepúlveda-Rodríguez, G., Rajan, S., Mitschke, M., Baird, E., & Vallejo-Marín, M. 2025. Increasing temperatures affect thoracic muscle performance in Arctic bumblebees. *Nature Communications*, 16, 9699.
4. Smith, D. B., Bernhardt, G., Raine, N. E., Abel, R. L., Sykes, D., Ahmed, F., Pedroso, I., & Gill, R. J. 2016. Exploring miniature insect brains using micro-CT scanning techniques. *Scientific Reports*, 6, 21768.
5. Schindelin, J., Arganda-Carreras, I., Frise, E., et al. 2012. Fiji: An open-source platform for biological-image analysis. *Nature Methods*, 9(7), 676–682.
6. Fedorov, A., Beichel, R., Kalpathy-Cramer, J., et al. 2012. 3D Slicer as an image computing platform for the Quantitative Imaging Network. *Magnetic Resonance Imaging*, 30(9), 1323–1341.
7. scibadger. *Bombus impatiens* photograph via iNaturalist. Licensed under CC BY-NC-ND.

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