Cool Muscles:
Storing Elastic Energy for Flight

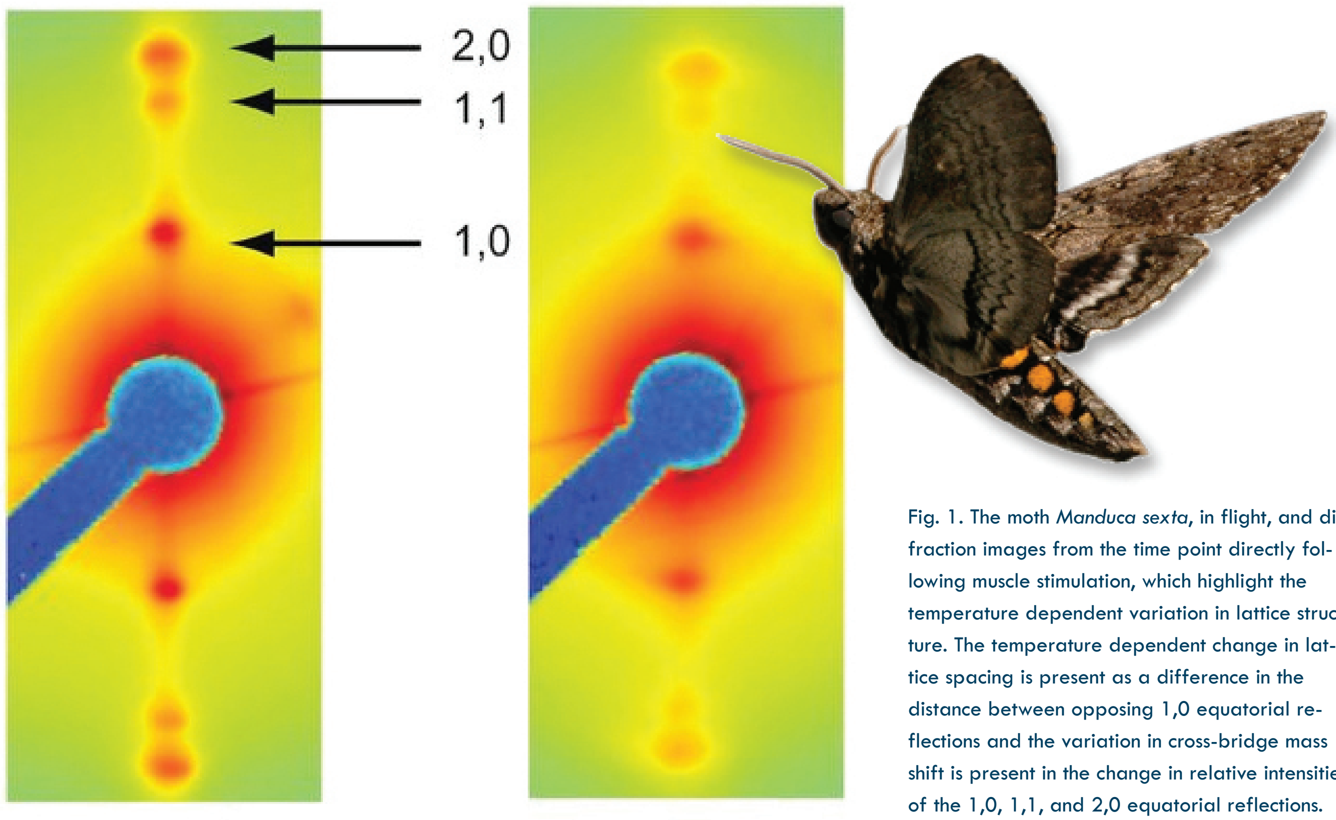

Fig. 1. The moth *Manduca sexta*, in flight, and diffraction images from the time point directly following muscle stimulation, which highlight the temperature dependent variation in lattice structure. The temperature dependent change in lattice spacing is present as a difference in the distance between opposing 1,0 equatorial reflections and the variation in cross-bridge mass shift is present in the change in relative intensities of the 1,0, 1,1, and 2,0 equatorial reflections. Diffraction patterns from N.T. George et al., Science **340**(6137), 1217 (7 June 2013).

Movie S1 @
http://www.sciencemag.org/content/suppl/2013/04/24/science.1229573.DC1/1229573s1.mov
View an animation consisting of 5-frame x-ray diffraction movies (on the right) paired with their respective mechanical measures of force and length (creating a work-loop, on the left), one from the 25° C condition and one from the 35° C condition. X-ray diffraction images from concurrent points in the contraction cycle highlight the temperature dependent variation in muscle lattice structure.

Flying has always fascinated humans, probably because we are so relentlessly Earthbound. One of the things that fascinates researchers who study flight is the question of how animals that do it can generate the energy required. Flying is an intensely power-hungry activity that is less than 10% efficient. Some studies have suggested that physical properties of the molecules involved in insect flight might contribute small amounts of energy to the flight "power grid" through potential energy savings in the form of elastic strain energy. Insight into this question has been provided by research completed at the Bio-CAT beamline 18-ID at the APS. These results provide important information about how flying species meet the energy needs of their powerful adaptation, new knowledge that may have implications for locomotion in general.

The study, by researchers from the University of Washington, the Illinois Institute of Technology, and Harvard University combined time-resolved small-angle x-ray diffraction with measurements of mechanical energy-exchange in the wing muscle of the moth *Manduca sexta* to create high-speed video of muscle motion at the molecular level. Their results show that temperature differences between the dorsal (top) and ventral (bottom) sides of the wing create an opportunity for elastic strain energy to be stored in the cooler regions of the muscle and then released during the transitions between contraction and relaxation to assist with the inertial power costs associated with accelerating and decelerating the wings.

The breakthrough in this work was the development of an apparatus that allowed the team to make high-speed x-ray diffraction measurements at the same time as they measured wing-muscle motion mechanics.

To do this, the researchers first fixed the body and flight muscle of the moth onto a "work-loop" apparatus that allowed them to stimulate muscle contractions in a controlled manner that simulated flight while measuring the forces of those contractions.

Next, the work-loop apparatus was aligned to the 18-ID x-ray beam and a custom shutter was installed to allow for high-speed measurements. The team recorded 5 diffraction images per wing-beat cycle, 1 every 8 msec, for 100 cycles at 25° C and at 35° C — temperatures that cover the range observed for *M. sexta* wing muscle.

The force generated during muscle contraction is known to result from the ratcheting of cross-bridge proteins along other muscle filament proteins. As the cross-bridge proteins bind, move, and release the filament, they generate force. As with all molecular interactions, the cross-bridges move faster at higher temperatures. These researchers had already shown that the temperature of the muscle on the dorsal and ventral sides of the moth wing can differ by as much as 6.9° C during flight.

By taking their x-ray diffraction images at 25° C and 35° C they were able to see what was going on at each temperature at the molecular level.

The x-ray diffraction data showed differences in the spacing of the molecules involved in muscle contraction depending on the temperature (Fig. 1). This indicated that the cross-bridges cycled faster at the warmer temperature and slower at the cooler temperature.

These results support a model in which the cross-bridges that drive the muscles on the warmer, underside of the wing cycle quickly during flight, while cross-bridges on the cooler, top side of the wing remain bound to filaments longer, building up strain until it is released as elastic energy as the muscle advances into its next phase of shortening or lengthening.

The combination of advanced technologies at the APS and the x-ray expertise of Tom Irving, Director of Bio-CAT and a collaborator on this project, made possible a new view of how temperature, strain, and molecular motors conspire to produce a range of functions in a single muscle: from an actuator to a spring, and provides important information about how flying species meet the energy needs of their powerful adaptation and may have implications for locomotion in general.

— Sandy Field

See: N.T. George[1], T.C. Irving[2], C.D. Williams[1,3], and T.L. Daniel[1]*, "The Cross-Bridge Spring: Can Cool Muscles Store Elastic Energy?," Science **340**(6137), 1217 (7 June 2013). DOI:10.1126/science.1229573
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