

Visualizing Vertebrates: New Methods in Functional Morphology

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Nearly all modern animal functional biology requires motion analysis. As many of the behaviors of greatest interest are very fast (e.g., prey capture and locomotion), image capture has always been of special concern. In the late 19th century, Eadweard Muybridge (and co-workers) solved this problem by rigging a series of still cameras to go off in sequence, ultimately recording the motions of many animals, including humans (Muybridge, 1887). This work provided a treasure trove of functional information, settling, for example, a long-standing dispute (as well as a famous, although possibly mythical, bet made by Leland Stanford) about whether all four feet of a horse leave the ground at any point during a gallop (they do; e.g., Clark, '31; MacGowan, '54a). Muybridge's methods ultimately led to his invention of the "Zoogyroscope" in 1879, the first motion picture projector (later called the "Zoopraxiscope"). However, a series of still cameras spread across some distance is impracticable for many animal movements of biological interest; hence, Muybridge's methods had limited scientific application. About the same time that Muybridge was making his photographs in California, brilliant French physician and scientist, Etienne-Jules Marey (inventor of many biomedical instruments), was perfecting his own system of motion picture capture. Significantly, however, he constructed a single camera that employed a rotating, emulsion-covered disc with an ingeniously synchronized mechanical shutter to capture 12 pictures per second (Marey, 1882a,b). Marey continued to develop various single-camera systems to perfect motion picture taking (or "chronophotography", as he called it) at increasing frame rates, which, unlike Muybridge, he applied specifically to the scientific understanding of animal locomotion (Fig. 1), particularly aerial locomotion in birds (something Muybridge had been unable to do with his multi-camera system; summarized in Marey, 1894, '02). It is a curious fact that, although Marey is well known to film historians (e.g., Gurnsheim and Gurnsheim, '55), his remarkable scientific work on animal mechanics is largely overlooked by modern, English-speaking functional morphologists.

Visualization and analysis of very rapid movements require a rapid frame rate (i.e., more "pictures per second") and short shutter speeds (the less time the shutter is open, the less motion blur). Marey's various motion picture cameras were capable of 1/720 sec or shorter shutter speeds—enough to freeze most motions quite well—but his film disc system limited the number of frames that could be taken (12–24) and therefore, the duration of a behavioral sequence that could be filmed. With the Eastman Company's invention of nitrocellulose film in 1889 (McGowan, '54b), the way was paved for faster frame rates and longer run times. In 1891, the Edison Company exploited this advance with their introduction of a new motion picture camera (the "Kinetograph") and a projector to display the films to audiences (the "Kinetoscope"). It is this basic form of technology that led to modern cinematography (Musser, '95) and ultimately, the scientific application of high-speed film to analysis of animal kinematics. However, film systems are cumbersome and have many limitations of speed, lighting, record time and resolution (and worse—one has to wait for the film to be developed before seeing the results!). During the last 25 years, film systems have been replaced by video systems (first analogue, then digital), and recently, these have achieved astounding frame rates and levels of resolution, making visualization of even the most fleet and fleeting of animal movements possible. As prices have come down, high-speed video systems have become standard equipment in most laboratories that study the dynamics of animal movement.

The story might end there except for one unfortunate fact—most animals are opaque. By definition, anatomists are primarily

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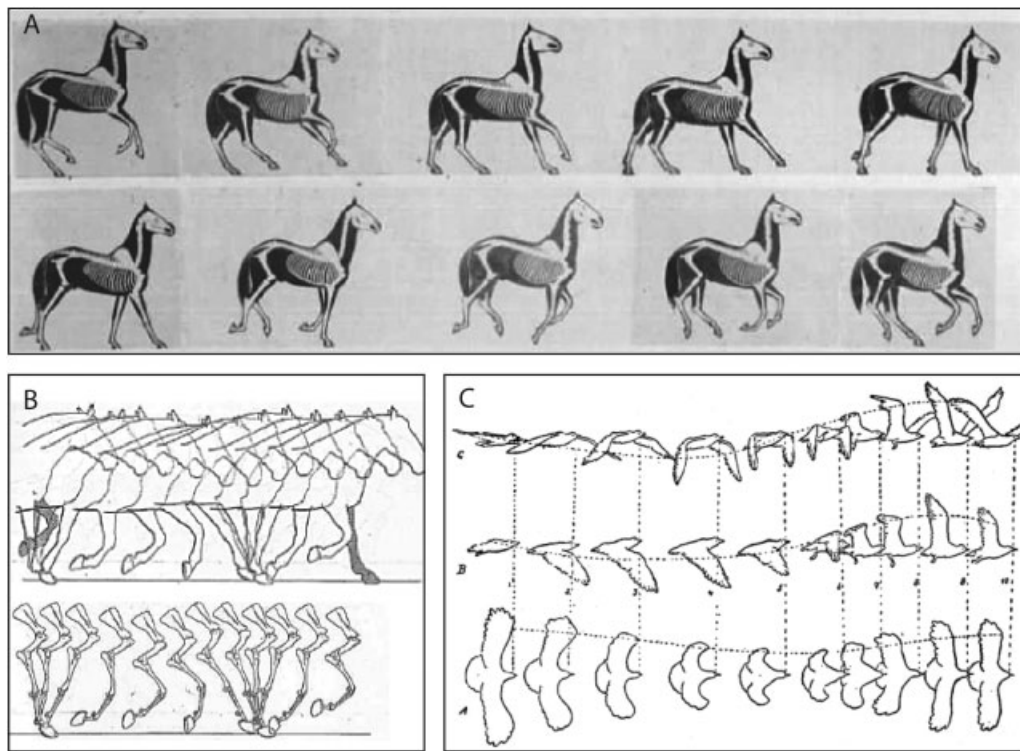


Figure 1. Examples of Etienne-Jules Marey's application of his own motion picture technology ("chronophotography") to studies of animal locomotion in the late 19th century. (A) Horse silhouettes traced from sequential motion picture frames, showing Marey's inferences about skeletal movements (from Marey, 1886). (B) Tracings from another chronophotographic sequence of horse locomotion, again showing detailed inferences about limb bone position (from Marey, 1898). (C) Marey was particularly interested in bird flight and took numerous motion picture sequences of various species in flight—something Muybridge was never able to accomplish. This particular figure is remarkable because it was constructed from motion picture sequences of a gull taken from three orthogonal points of view (at the same frame rate) and then matched frame-by-frame according to the phase of the wing beat cycle (from Marey, 1890). Marey made significant advances in the three-dimensional reconstruction of flight trajectories and kinematics (e.g., Marey, 1869, 1890, 1894).

interested in the inner bits of their subjects and must usually resort to dissection to observe them. However, dissection and animal movement are, one might say, inimical; hence, new methods were needed to visualize the motion of parts hidden within living, moving organisms. For example, many studies rely on the use of the surface markers to delimit joint segments, permitting one to create stick figure models that serve as proxies for skeletal movements. Skin, however, is usually only loosely adhered to the underlying skeleton and the correspondence between surface markers and actual bones is often poor (see Brainerd et al., 2010; Gatesy et al., 2010). To address this problem, x-ray or fluoroscopy machines are often combined with high-speed film and video systems so that the *in vivo* motions of bones and other hard tissues can be observed directly. As groundbreaking and useful as these studies have been, they all suffer from the problem of parallax, owing to the fact that a fluoroscopic image is a two-dimensional projection of a complex (and complexly moving) three-dimensional structure. Many

biologically important behaviors involve not only planar movement but also potentially, simultaneous rotation, yaw and pitch! The "flattening" of an x-ray image makes interpretation of such bone movements difficult or impossible, especially when the displacements are small, yet functionally critical (e.g., cranial kinesis in a lizard during prey capture or the mandibular orbit in a chewing mammal). Consequently, only purely planar sequences provide accurate measurements of skeletal movements, and such sequences are hard to obtain and often do not reflect the real complexity of natural animal movements. Surgical implantation of radio-opaque markers onto bones has helped to make quantification of skeletal movements more precise, but it does not overcome the problem created by the loss of dimensionality.

The obvious but technically challenging solution to these problems is to employ two (or more) video or videofluoroscopic cameras simultaneously in order to obtain *x*, *y* and *z* coordinates of skeletal landmarks or markers. Skeletal movements can then be reconstructed in three dimensions. The irony of these methods

is that oftentimes the complexity of the data requires that its dimensionality be reduced for representation in two-dimensional kinematic plots! Ideally, one would like to create accurate, three-dimensional images of actual animals that reveal their in vivo skeletal movements from any point of view. Remarkably, this is exactly what has been accomplished by two research teams at Brown University, whose methods are formally described for the first time in the pages of this issue (Brainerd et al., 2010; Gatesy et al., 2010). The three-dimensional visualization of vertebrate skeletal movements achieved by these workers using different but related methods are nothing short of amazing (see supplementary videos). Both teams employ a combination of high-speed videofluoroscopy and detailed, three-dimensional reconstructions of skeletons using high-resolution computed tomography (CT) scans to create highly precise animations of moving skeletons. Because these three-dimensional animations are created digitally, they can be viewed from any angle or desired perspective. Gatesy et al. adapt a traditional, commercial animation technique known as “rotoscoping” to scientific ends. This method uses both light and x-ray video-generated images of living animals to serve as “constraints” on which digital “marionettes” of the animals’ skeletons are superimposed frame by frame using animation software. The result is a highly accurate, three-dimensional, digital model of the entire skeleton in motion that is based directly on the detailed anatomy of individual bones and joints, as well as light and x-ray films of the same animals in motion. As such, movement of some skeletal elements that are not directly visualized in the x-rays can be accurately predicted and shown in the animations, as can movements in planes not filmed. Indeed, the method has the advantages of requiring only a single x-ray unit and no invasive surgery for marker placement. Brainerd et al., in contrast, take a more direct approach in applying two x-ray units and implanted skeletal markers to generate highly precise, three-dimensional, skeletal movement data (x, y and z coordinates for each marker through time). The three-dimensional x-ray data are then combined with high-resolution CT scans of the skeleton of the same animal used in the high-speed video analysis (following sacrifice). The digital CT skeletal model can then be precisely aligned with the kinematic data derived from the videofluoroscopy using marker positions to produce three-dimensional animations of the moving skeleton viewable from any angle. Because both methods employ actual skeletal morphology and arthrology (joint anatomy) to inform their kinematics, the animations can reveal accurate and sometimes unpredictable, or even surprising, results.

The *Journal of Experimental Zoology A* is exceptionally pleased to present these two groundbreaking articles to the scientific community. Together, they represent an important, even pivotal, advance in the field of vertebrate functional morphology and the authors are to be congratulated both for their efforts and for making the fruits of their years of labor

available for implementation in other labs around the world. These articles highlight the importance of publishing significant advances in methodology in any field as an aid to moving the discipline forward. Such articles can lead to critical and unforeseen breakthroughs, and even new scientific horizons (take, for example, the polymerase chain reaction). *Journal of Experimental Zoology Part A* encourages the submission of articles that offer new methods and advances in a variety of disciplines within integrative biology.

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