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# Hindlimb muscle anatomical mechanical advantage differs among joints and stride phases in basilisk lizards

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## ABSTRACT

The vertebrate musculoskeletal system is composed of skeletal levers powered by muscles. Effective mechanical advantage (EMA) and muscle properties influence organismal performance at various tasks. Anatomical mechanical advantage (AMA) is a proxy for EMA that facilitates the study of preserved specimens when many muscles or many species are of interest. AMA is the quotient of in-lever to out-lever length, and quantifies the force–velocity trade-off of a lever, where high AMAs translate into high force, low velocity levers. We studied AMAs, physiological cross-sectional areas (PCSAs), fiber lengths, and fiber widths for 20 hindlimb muscles of the lizard *Basiliscus vittatus*, moving the hip, knee, and ankle during both the stance and swing phases of the stride. We tested the hypotheses that muscles moving proximal limb joints, and those active during stance, would have characteristics that maximize force. We also tested whether adults had more force-optimized levers than juveniles to compensate for higher body mass. We found no differences between adults and juveniles, but found differences among joints and between stride phases. AMAs were lowest and PCSAs highest for the knee, and PCSA was higher for stance than swing muscles. Fiber width decreased distally, but did not differ between stride phases. Fiber length of stance muscles decreased distally and was highest for swing muscles of the knee. Our findings show that different muscle and lever characteristics allow the knee to be both force- and velocity-optimized, indicating its important role in locomotion.

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## 1. Introduction

Animals rely on their musculoskeletal system to enable them to accomplish various tasks (Hildebrand et al., 1985; Huijing, 1998). Many muscles are involved in multiple tasks and their properties frequently represent a compromise between those tasks (Gans and De Vree, 1987; Foster and Higham, 2014). The action of a given muscle depends on its moment arms and attachments to the skeleton (Huijing, 1998). The properties of muscles and the skeletal lever systems that they move determine locomotor capacity and reflect the animal's ecology (Biewener, 1989; Westneat, 1994; McHenry and Lauder, 2006; Scales et al., 2009; Higham et al., 2011; Holzman et al., 2011a). Ultimately, since different muscles move different joints, and may be active during different phases of a behavior (Gregersen and Carrier, 2004; Foster and Higham, 2014), they may have different demands placed on them depending on the size of the animal (Carrier, 1983, 1996; Herrel and Gibb, 2006). One would expect, therefore, that muscle and lever system properties

vary according to the functional demands they experience. Here we study anatomical properties of muscles and lever systems in the hindlimb of the basilisk lizard (*Basiliscus vittatus*) to test if they differ among joints and between stance and swing phase muscles.

Muscles are composed of dissectible fibers, which are bundles of myofibrils, containing sarcomeres, the serially arranged contractile units of the myofibrils (Hildebrand et al., 1985; Gans and De Vree, 1987). The contractile properties of muscles are affected by fiber lengths, widths, angles, and types. Longer fibers have more sarcomeres in series, and so have higher contraction velocities and displacements, but fiber length is independent of force (Sacks and Roy, 1982; Gans and De Vree, 1987; Azizi et al., 2008). Wider fibers have more sarcomeres in parallel and produce more force (Gleeson, 1983; Gans and De Vree, 1987). This pattern scales up to the whole muscle in that muscles with higher physiological cross-sectional areas (PCSAs) also produce more force (Sacks and Roy, 1982; Powell et al., 1984; Gans and De Vree, 1987). The angle of muscle fibers relative to their line of action influences both force and contraction velocity in the direction of action (Gans and De Vree, 1987). Pennate muscles have short fibers oriented at an angle to the line of action, reducing velocity but increasing total force because more fibers are placed in parallel (Sacks and Roy, 1982; Huijing, 1998).

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The contractile properties of muscles are also influenced by their fiber types (Gleeson et al., 1980; Bonine et al., 2005), but this is one factor not dealt with here. The physiology and architecture of muscles establish force–velocity trade-offs that have implications for organismal performance at various tasks (Gans and De Vree, 1987; Bonine et al., 2005).

Another way in which musculoskeletal systems vary is in the properties of their lever systems, influencing organismal task performance above the cellular level (Gans and De Vree, 1987; Biewener, 1989). A lever rotates around a fulcrum, and the in-lever and out-lever are the distances from the fulcrum to the in-force applied by a muscle and the out-force produced by the lever (Hildebrand et al., 1985). Lever systems are governed by the formulae

$$F_{out} = \frac{F_{in}L_{in}}{L_{out}} \quad \text{and} \quad V_{out} = \frac{V_{in}L_{out}}{L_{in}},$$

where $F$ is force, $L$ is the lever length, and $V$ is velocity for the in- and out-levers (Hildebrand et al., 1985; McHenry and Lauder, 2006). From these equations, it is apparent that changing the relative lengths of the in- and out-levers affects out-force and out-velocity inversely. Conveniently, one can define the lever mechanical advantage (MA), the inverse of gear ratio (GR), as $MA = 1/GR = L_{in}/L_{out}$, where a lever with a low MA or high GR produces low-force, high-velocity movement (Gans and De Vree, 1987; Biewener, 1989, 1990; Carrier et al., 1998; McHenry and Lauder, 2006).

In musculoskeletal systems, gearing occurs at multiple levels, including the levers of the myosin heads, the levers associated with the architecture of muscles (pennation), and the levers of the skeleton being moved by muscles (Azizi and Brainerd, 2007; Azizi et al., 2008). At the level of the skeletal levers, which is our focus here, MAs have been calculated in two ways. Effective mechanical advantage (EMA) calculates the in-lever as the perpendicular distance from the center of the joint to the line of action of the muscle, and the out-lever as the perpendicular distance from the joint to the ground reaction force (GRF) vector (Gans and De Vree, 1987; Biewener, 1989; Carrier et al., 1998; Gregersen and Carrier, 2004). EMA allows one to precisely model gearing during the behavior of an animal, accounts for the non-independence of EMA estimates among connected joints, and provides data on the dynamic nature of gearing as a joint moves (Biewener, 1989; Carrier et al., 1998; Gregersen and Carrier, 2004). However, the EMA cannot be applied to preserved specimens, and is labor-intensive, limiting its utility for assembling large comparative datasets. It is also typically applied on a joint-specific as opposed to muscle-specific basis (Carrier et al., 1998; Gregersen and Carrier, 2004; Azizi and Brainerd, 2007).

Anatomical mechanical advantage (AMA) is a static estimate of the actual MA whose accuracy depends on the behavior being studied (Young, 2009; Fellmann, 2012). AMA is more easily measured than EMA because it does not require live specimens and GRF or kinematic data. AMA calculates the in-lever as the distance from the center of the joint to the insertion of the muscle, and the out-lever as the length of the bone being moved by the joint (Carrier, 1983; Hildebrand et al., 1985). The AMA has the advantages of easily being applied on a muscle-specific basis, and to large numbers of species and specimens, facilitating evolutionary studies (Hildebrand et al., 1985; McHenry and Lauder, 2006). However, the AMA treats gearing as static and does not take kinematics, kinematic chains, or behavior into account (Young, 2005, 2009).

Here, we compare AMA among hindlimb muscles that rotate the hip, knee, and ankle during the stance and swing phases of the stride in the lizard *Basiliscus vittatus*. This species is a good candidate for our study because individuals exhibit high levels of locomotor performance, being bipedal specialists that run quickly on both land and water (Snyder, 1949, 1962; Hsieh, 2003; Hsieh and Lauder, 2004). They need to generate large forces during these locomotor behaviors (Glasheen and McMahon, 1996; Hsieh and Lauder, 2004). They also increase in size by an order of magnitude (Glasheen and McMahon, 1996), allowing us to investigate whether AMAs change through ontogeny, something that has been shown to happen in various animals, possibly to compensate for decreased muscle strength and relative muscle mass of juveniles relative to adults (Goldspink, 1980; Carrier, 1983, 1996; Young, 2009).

Despite considerable work on mechanical advantage in vertebrate limbs, there are few studies that simultaneously consider a large number of muscles that differ functionally, allowing us to test several novel hypotheses. First, we test the hypothesis that muscle properties and AMAs differ among joints of the hindlimb. We expect that more proximal joints will be optimized for force output, while more distal joints will be optimized for velocity because proximal joints move a heavier proportion of the limb than distal joints. This is exacerbated by proximal muscles tending to be largest (Maloiy et al., 1979). Hence, we expect muscles powering proximal joints to have higher AMA and PCSA, and shorter, wider fibers than distal joints (Carrier et al., 1998; Azizi and Brainerd, 2007; Li et al., 2012). Second, we test whether muscles active during stance phase will be force-optimized, while those active during swing phase of the stride will be velocity-optimized. The force needed to propel an animal during locomotion is exclusively generated during stance (Higham et al., 2011), so stance phase muscles should be force-optimized. Third, we test whether muscle and lever system properties of juvenile animals differ from those of adult animals. Juvenile animals sometimes exhibit relatively higher locomotor performance than adults (Herrel and Gibb, 2006) and may have higher mechanical advantage to compensate for relatively weaker and smaller muscles (Carrier, 1996). However, adults may need to generate more force to compensate for their disproportionately greater weight (Biewener, 1989). Finally, we wish to highlight the utility of AMA as a viable approach to studying lever properties when the goal is to study large numbers of muscles in large numbers of specimens or species.

## 2. Materials and methods

### 2.1. Study animals

We used 15 *B. vittatus* specimens for limb muscle dissections, obtained from a commercial dealer. The size of the animals followed a bimodal non-overlapping distribution, with six juveniles (snout–vent length (SVL) mean = 70.5 mm, range: 62.4–80.5 mm) and nine adults (SVL mean = 119.0 mm, range: 104.7–138.9 mm). We euthanized the animals using an overdose of isoflurane gas (AVMA, 2000). We then fixed them for three days using 10% neutral buffered formalin in standardized position with the thigh perpendicular to the body, and the knee and ankle joints at 90° to ensure that muscles were comparable in terms of length and appearance (Pisani, 1973; Sacks and Roy, 1982; Herrel et al., 2008). Using this standardized orientation of the limb segments will facilitate comparison to species studied in the future. These joint angles fall within the range of angles that lizards use during locomotion (Irschick and Jayne, 1998; Foster and Higham, 2012), including basilisks running on water (Hsieh, 2003). Finally, we soaked specimens in water for 24 h to remove excess formalin and preserved them in 70% ethanol. All of these procedures are standard and were approved by Clark University's Institutional Animal Care and Use Committee.