

BIOMECHANICS OF TERRESTRIAL LOCOMOTION IN BATS

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This dissertation concerns the way in which bats move on the ground. Chapter one is a literature review on the subject, from an evolutionary perspective, that includes contributions from this thesis.

In chapter two, I test an hypothesis frequently used to explain the poor crawling abilities of bats compared with mammals that do not fly. According to that hypothesis, most bats shuffle awkwardly because their hindlimbs are too long and slender to support their body weights, but vampire bats walk well because their hindlimbs are more robust than those of other bats. I used force plates to test a prediction of the hindlimb-strength hypothesis that the peak hindlimb forces of walking vampire bats should be greater than the forces exerted by the legs of poorly crawling bats. I found that shuffling bats (*Pteronotus parnellii*) exert larger hindlimb forces than walking vampire bats do (*Desmodus rotundus*, *Diaemus youngi*). Additionally, I used a simple engineering model of bone stress to demonstrate that the hindlimbs of vampire bats fall within the range of shapes seen in bats that do not walk well. These results do not support the hindlimb-strength hypothesis.

In chapter three, I describe the running gait of Common Vampire Bats (*D. rotundus*). At low speeds, these bats use a lateral sequence walking gait, similar to those of other tetrapods, but switch at higher speeds to a bounding gait that is powered by the forelimbs. This gait is unique to vampire bats,

and appears to be independently evolved from the running gaits of other tetrapods.

In chapter four, I compare the kinematics of locomotion in Common Vampire Bats to those of another terrestrially adept species, New Zealand short-tailed bats (*Mystacina tuberculata*). The latter use a lateral sequence walk similar to that of *D. rotundus* and other tetrapods, but do not perform the bounding run. Using force plates to examine the kinetics of their single kinematic gait, I found that the gait of *M. tuberculata* is a kinetically run-like, and does not shift from a kinetic walk to kinetic run with increased speed the way the gaits of some other animals do.

BIOGRAPHICAL SKETCH

Daniel Kenneth Riskin was born to Mary W. Walters and Gerald A. Riskin on July 7th, 1975. His brother Matthew was born in 1977 and his sister Raquel in 1984. Dan grew up in Edmonton, Alberta.

Dan's involvement with bats began at a lake northeast of Edmonton, where, as a teenager, he first noticed them circling high above the campfire and was absolutely blown away to be seeing bats at all. In high school, Dan read a popular book on bats by M. Brock Fenton, and started annoyingly repeating bat facts to everyone around him. This is a habit Dan has yet to break.

In his first undergraduate year at the University of Alberta (1993), Dan took a truly inspiring introductory zoology course from Reuben Kaufman that led him to focus his studies on biology. At the U of A, Dan was fortunate enough to take particularly memorable courses from Mike Caldwell, Dick Fox, Jan Murie, Joe Nelson, Curt Strobeck, and Lawrence Wang, among others. In his fourth year, Margo Pybus, of Alberta Fish and Wildlife, kindly presented Dan with data she had collected on bats using the sides of buildings in Alberta as day roosts, which Dan analysed to produce his first scientific paper.

In the summer of 1996, Dan went to Montreal, where he was employed for the summer by Robert Carroll at the Redpath Museum (McGill University) to research the diversification of eutherian mammals at the Cretaceous-Tertiary boundary of North America. Dan was thrilled to learn

later that year that his work had been included in a book by Dr. Carroll on vertebrate evolution.

After graduation, Dan took a “real-world” job in marketing for Lone Pine Publishing in Edmonton, which he neither enjoyed, nor was particularly good at. However, the natural history focus of that organization kept his skewer in the great fondue of science, and at the urging of his good friend John Clare, Dan applied for a scholarship to do graduate school. He won the award, bid farewell to the real world, and dove straight back into the academic one, this time at York University in Toronto in the lab of that first book’s author, Brock Fenton.

At York, Dan had the opportunity to collaborate on two ecology projects in Belize, and conducted his own research in Costa Rica on the mechanisms by which Spix’s Disk-winged Bats adhere to smooth leaves. This turned out to be much more fun than selling books over the phone. Dan received his M.Sc. in 2000.

Afterward, Dan moved to Victoria, BC, where he taught biology at Camosun College, and found a passion for teaching that he hadn’t recognized at York. This got him thinking about getting back into academics, and when the North American Symposium on Bat Research happened to occur down the street from his apartment in 2001, Dan attended a talk by Bill Schutt on terrestrial locomotion by *Cheiromeles torquatus*, went for beer with John Ratcliffe, and realized he’d gotten off the bus a few stops early. Later that week, Dan applied to the zoology program at Cornell University to work with John Hermanson, and was ultimately accepted.

Dan has enjoyed his roles as teacher and researcher at Cornell, and hopes to further pursue these passions upon graduation.

This thesis is dedicated to my brother, whom I once underappreciated.

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As committee member, David Winkler has brought a wealth of knowledge about the evolutionary and ecological questions that interest me. He gave me an exceptionally interesting reading list for my A-exam and helped me to see my projects differently through thoughtful questions, discussions, and suggestions during our meetings. My dissertation is far better for his influence.

One of my favourite things about working at Cornell has been the opportunity to spend time with Andy Ruina. As a committee member, he has guided me through a curriculum of engineering classes that have absolutely changed the way I think about animal movement. He has also discussed my research with me at a level of detail that reflects a sincere interest in my education and a generous investment of time. I am very grateful to him for his involvement in my education.

My advisor, John Hermanson has been outstanding. I probably would have been happy to just go on reading about nothing but bats until I went blind, but John has pushed me to learn the neurobiology, physiology, histology, and anatomy that are necessary to understand the literature

surrounding my field of study. He has also ushered me into the North American biomechanics community, increasing the breadth of questions I address in my work. The end result of all this pushing has been a body of work that I'm very happy with, and a curiosity for things non-bat that will keep me from narrowing my focus too much in the future.

John has helped my research in every way possible. He's offered suggestions, purchased equipment, spoken on my behalf with Trinidadian government and University officials, helped me with data collection, given me hockey tickets, and even let me borrow his house. Perhaps the most exciting moment of my academic career was witnessing that first vampire bat break into a bounding run. I am very glad that John was in the room with me to see it happen. He's a great thinker and a good friend.

In the history of PhDs, no grad student can possibly have had the good fortune I had in terms of a field assistant. Gerry Carter works incredibly hard, and inspires me with a passion for bats that even surpasses my own. He has helped my PhD in more ways than I can possibly mention here, and I thank him especially for that.

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CHAPTER 1: INTRODUCTION: THE EVOLUTION OF TERRESTRIAL LOCOMOTION BY BATS (MAMMALIA: CHIROPTERA)

Summary

Despite several examinations of bat anatomy, many with emphasis on quadrupedal locomotion, the precise mechanisms that prevent most bats from walking well are still not clearly understood. Those taxa that walk well appear able to do so as the result of enlarged forelimb musculature, fatigue-resistant forelimb muscle fibres, and perhaps most importantly, enhanced hindlimb mobility.

The evolutionary sequence that led bats from a terrestrially agile ancestor to their current abilities is most likely intertwined with the sequence that made them able to fly. It is likely that the head-down roosting posture of bats evolved in their non-volant arboreal ancestors. Gliding forms with pendulous roosting habits would have undergone weaker selection for terrestrial agility than for aerial agility, so some adaptations for flight, especially related to the orientation of the hindlimbs, evolved at the expense of crawling ability. From a poorly crawling ancestor, the vampire bats and short-tailed bats have independently evolved terrestrial agility afresh, each in response to the selective pressures associated with its unique ecological niche.

Introduction

The bats make up more than 20 percent of mammalian species, occupy a broad range of dietary niches, and are found everywhere there is land, with the exception of Antarctica and a few isolated islands (Simmons, 2005). Aside from the birds and pterosaurs, bats are the only vertebrates to have evolved powered flight. This mode of locomotion is energetically expensive (Ward et al., 2004), and bats are remarkable athletes, both in terms of endurance and agility. For example, the Mustached Bat, *Pteronotus parnellii*, weighs only 10 to 20 g, and beats its wings in flight constantly for 5 to 7 hours per night. It performs acrobatic twists and rolls in the echolocation-driven pursuit of aerial prey, that it locates, captures, and ingests on the wing (Goodwin, 1970). At the end of each flight, the bat performs a final flip to put its toes above its head in order to perch head-down from the ceiling of the roost.

This degree of aerial agility is commonplace among bats, but this dexterity ceases when a bat finds itself on the ground. Having fallen from a roost, or collided with another object during flight, many bats drag their abdomens across the ground by a series of asymmetrical limb movements, whereas others appear unable even to take even a single step (Vaughan, 1959). Bats from a few phylogenetically isolated taxa walk well, but compared with small mammals of similar size, even these bats are somewhat slow and fairly easy to capture by hand from the ground (Lawrence, 1969).

If there were no associated cost, the ability to walk on the ground would confer a selective advantage to any species of bat. It would enable them to escape danger after an accidental fall, and would also permit them to exploit a more broad range of food resources. As evidence, consider the short-tailed bats, *Mystacina tuberculata*, of New Zealand, which are exceptionally good walkers, and spend around 30% of their foraging time on the ground (Daniel, 1976). They eat fruit, nectar, and flower fragments from over a dozen plant species, a broad range of insects (Lepidoptera, Coleoptera, Diptera, Blattodea, Neuroptera, Hymenoptera, Orthoptera, Hemiptera, and Thysanoptera), spiders (Araneae), mites (Acari), harvestmen (Opiliones), centipedes and millipedes (Myriapoda), and amphipods (Arkins, 1999). Because they forage terrestrially, *M. tuberculata* may have the most phylogenetically diverse diet of any bat (Lloyd, 2001). Whereas many other insectivorous species increase the breadths of their diets by gleaning prey from the ground (Johnston and Fenton, 2001; Ratcliffe and Dawson, 2003), the evolutionary transition to quadrupedal foraging is an extreme rarity among bats. Terrestrial mobility would enable bats to take advantage of seasonal changes in food abundance, and would permit greater niche partitioning of habitats among species. However, bats are conspicuously absent from terrestrial niches, even in the tropics where the number of bat species is high, and their dietary niches are diverse (Arita and Fenton, 1997). With so much to be gained from foraging terrestrially, why are there so few bats that walk well? Many birds forage terrestrially, even though they retain the ability to fly, so what prevents bats from widespread terrestrial agility?

Variability in terrestrial agility among bats

A flying bat runs the risk of accidentally falling on the ground, and every bat appears to be equipped with some strategy for dealing with this event, even though most bats avoid the ground for the majority of their lives. There are over 1,100 bat species, assigned to 18 families (Simmons, 2005), and of those bats so far surveyed, the abilities of each species can be placed into one of three categories: bats that cannot walk at all; bats that shuffle awkwardly using a series of erratic movements; and bats that walk well (Vaughan, 1970).

Several bats are thought to be unable to walk at all. These include the natalids *Natalus stramineus* (Vaughan, 1970) and *N. tumidirostris* (Riskin et al., 2005), several phyllostomids, such as *Macrotus californicus* (Vaughan, 1959) and *Leptonycteris* sp. (Dietz, 1973), and at least some rhinolophids, hipposiderids (Lawrence, 1969), and mormoopids (*Mormoops megalophylla*; personal observation). When placed on the ground, these animals take flight immediately by violently slapping their wings against the ground to launch into the air. Although crawling might be simply because bats of these species prefer not to, it seems likely that they are prevented from performing terrestrial locomotion by some aspect of their morphologies (Vaughan, 1970). Of those species surveyed, bats that cannot walk as adults do walk as juveniles (Dietz, 1973). This could reflect the increased importance of non-aerial locomotion to animals that can not yet fly, or it may simply be a byproduct of the ontogenetic progression toward an adult form.

When grounded, many bats use a series of asymmetrical movements to push themselves across the substrate. Because those motions are so variable, they are difficult to describe accurately. Published accounts of this

kind of shuffling include those of *Eptesicus serotinus* (Vespertilionidae; Lawrence, 1969), *Artibeus* sp. (Phyllostomidae; Dietz, 1973) and *P. parnellii* (Mormoopidae; Riskin et al., 2005). Although less than 5% of bat species have been observed carefully, the general impression among bat morphologists is that the majority of bat species manoeuvre poorly on the ground (Vaughan, 1970), and it is this general trend among bats that makes the agility of a few nimble species, such as the vampire bats, so remarkable (Altenbach, 1979).

The Common Vampire Bat (Phyllostomidae: *Desmodus rotundus*) is probably the most terrestrially agile of all the bats. It can walk forward, backward, and sideways (Altenbach, 1979), run with a bounding gait at speeds greater than $1.1 \text{ m}\cdot\text{s}^{-1}$ (Riskin and Hermanson, 2005), and jump directly into flight in under 0.03 s (Schutt et al., 1997). The other vampire bats (*Diaemus youngi* and *Diphylla ecaudata*) possess similar agility, though they do not run (Riskin et al., in press), and seem more adapted to arboreal climbing than to terrestrial walking (Schutt, 1998). The distantly related Short-tailed Bats of New Zealand (Mystacinidae: *M. tuberculata*) also walk well, and these four species taken together are generally considered more terrestrial than any other bats (Altenbach, 1979; Dwyer, 1960a). However, with descriptions lacking for so many species, this statement remains unverified. Several vespertilionids, such as *Antrozous pallidus*, *Nyctalus noctula* and *Myotis septentrionalis*, move very quickly on the ground (Lawrence, 1969; Orr, 1954; J. M. Ratcliffe, personal communication), as do a great number of molossid bats (Dietz, 1973; Lawrence, 1969; Schutt and Simmons, 2001; Vaughan, 1959). Anecdotal evidence and morphological analyses suggest that some molossids, such as *Cheiromeles torquatus* and

C. parvidens of Indonesia and the Philippines, and *Tadarida australis* of Australia, are perhaps even as terrestrially adept as *M. tuberculata* (Freeman, 1981; Mills et al., 1996; Schutt and Simmons, 2001), however no kinematic descriptions have been reported in the literature for those species.

Hindlimbs and the evolution of flight

Although historically there was some debate as to whether flight evolved once or twice among mammals (Pettigrew, 1986), there is now general consensus that the bats are monophyletic, and that flight evolved just once (Teeling et al., 2005; Van den Bussche and Hoofer, 2005). It is therefore likely that the reduction of terrestrial agility that characterizes the bats in general also evolved once, perhaps in association with that transition to aerial locomotion.

Fossils clarifying the postcranial anatomy of the mammals that gave rise to bats have not been uncovered, but most authors suspect that bats evolved flight 'trees-down,' from an arboreal ancestor that evolved gliding, then later gave rise to animals capable of powered flight (Clark, 1977; Norberg, 1985; Padian, 1987; Schnitzler et al., 2003; Simmons and Geisler, 1998; Speakman, 2001). The ability to glide by means of membranes stretched between the limbs has evolved independently several times among arboreal mammals, including Rodentia (flying squirrels), Dermoptera (flying lemurs), several times among Marsupiala (sugar gliders), but only the bats acquired powered flight (Lindhe Norberg, 2002; Scholey, 1986). This demonstrates that flight is not an inevitable consequence of gliding, and that gliding itself can represent an adaptive plateau (Speakman, 2001). However,

the ability to hawk insects in flight is thought to be the 'key innovation' that gave bats the ability to explode in diversity during the Eocene (Simmons and Geisler, 1998; Teeling et al., 2005), and was obviously an essential step in their evolutionary history.

Bats are the only mammals that fly, so their limbs are different from those of other mammals. The elongation of the forelimb bones to support the membranous wings is striking, but modifications of the hindlimbs are also important. During both flight and roosting, the femora of bats extend laterally or caudally (out to the side, or backward), and are rotated on their long axes so that the knees point laterally and dorsally, whereas the plantar surfaces (soles) of the hind feet face ventrally (Fig. 1). This permits the claws to grip when a bat hangs head-down with its chest against a surface, and is also the orientation of the hindlimbs when bats crawl on the ground. This rotation, or 'reversal,' of the hindlimbs prevents bats from positioning their hindlimbs during walking the way most mammals do, and is thought to be one of the main reasons that most bats walk poorly (Vaughan, 1959, 1970).

However, hindlimb reversal is not unique to bats, and is performed by several other mammals that walk perfectly well. Indeed, the ability to point the toes caudally has evolved independently several times among arboreal and climbing mammals, including members of the Multituberculata (Jenkins and Krause, 1983), Marsupialia, Carnivora, Edentata, Primates, Rodentia, and Scandentia (Jenkins and McClearn, 1984). Importantly though, the hindlimb reversal of these non-bat mammals occurs due to specializations of the ankle, especially at the talocrural and subtalar joints, whereas in bats the

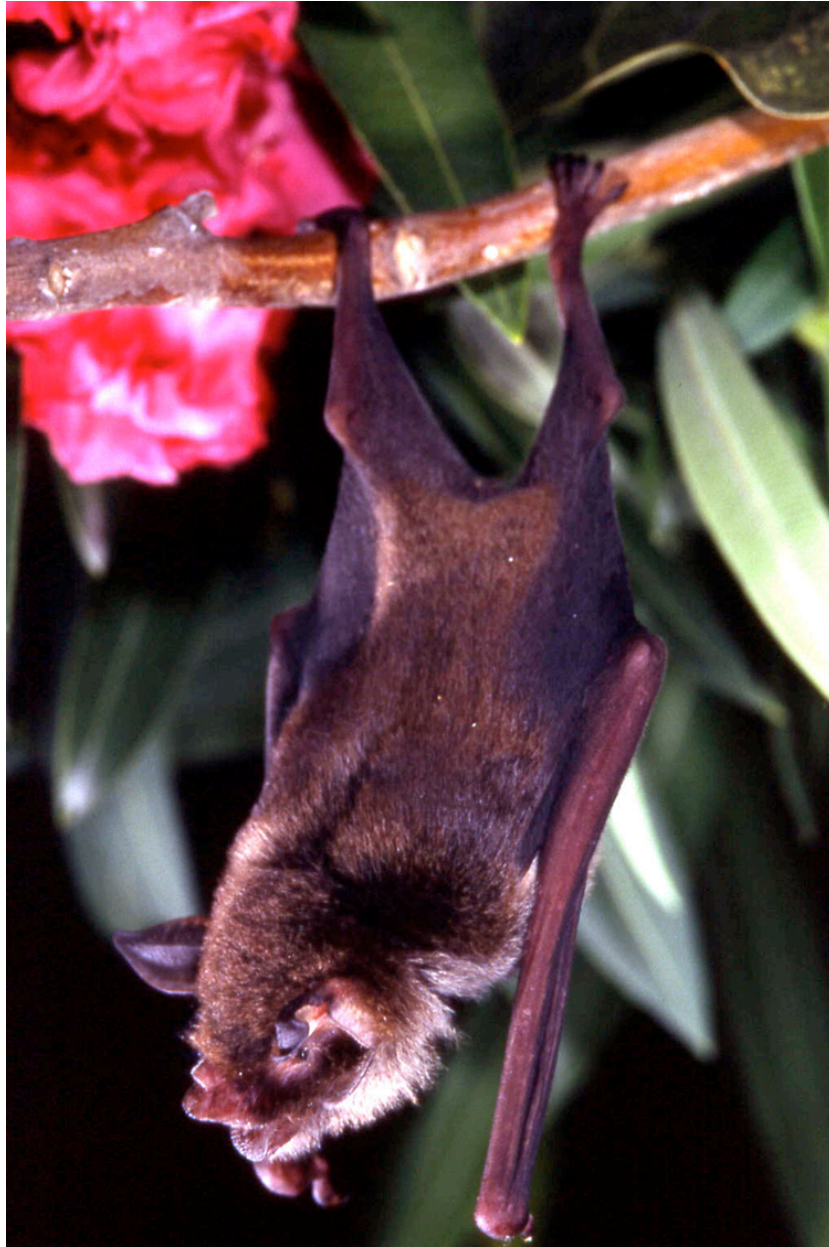


Figure 1: Roosting posture of Parnell's Mustache Bat (*Pteronotus parnellii*), that demonstrates the hindlimb orientation of bats. Note that the knees point laterally and dorsally whereas the soles of the feet face ventrally. This is typically referred to as 'hindlimb reversal,' because the toes of bats point in the opposite direction from those of a cat or dog, for example. This prevents bats from using their legs for walking the way most mammals do, and is thought to be one of the main reasons bats walk poorly. Photograph by M. Brock Fenton.

rotation principally occurs at the hip. The reason for this difference is unknown, but its result is that while other mammals generally return the hindlimbs to the more typical mammalian orientation when walking on the ground, bats cannot (Jenkins and McClearn, 1984; Vaughan, 1970). Hindlimb reversal has the additional consequence that it enables animals to hang bat-like from horizontal supports, as evidenced by squirrels (Rodentia), kinkajous (Carnivora) (Jenkins and McClearn, 1984), and the bats themselves.

Hindlimb reversal is common among quadrupedal mammals that manoeuvre arboreally. It permits the squirrel in your yard to descend a tree head first, while the cat that chased it up there sits helplessly on a branch. This is explained by a free-body diagram of a quadrupedal animal grasping a vertical surface with two limbs at an upper point of contact and two limbs at a lower point of contact (Fig. 2). Because the gravitational force vector at the animal's centre of mass (some distance from the surface) is not aligned with the normal force vector (where the limbs grasp the surface), a torque is created that pulls the animal away from the surface at the upper limbs. To resist falling, an animal must be able to grip the surface at the upper point of contact (Alexander, 2003). From a head-down posture, mammals with cat-like hindlimbs cannot cling to the trunk with their hind claws, because their claws hook away from the surface (and are not reversible themselves), but mammals with reversed hindlimbs can.

Based on the phylogenetically widespread trend toward hindlimb reversal among arboreal mammals, it seems reasonable that the ancestors to bats evolved the reversed hindlimb as they became adapted to an

arboreal habitat – before flight. Once hindlimb reversal was achieved, the proto-bat could have adopted the pendulous roosting posture that typifies modern forms. Certainly, a hanging posture would have had important consequences to terrestrial locomotion, and although it has been overlooked in several reviews (Arita and Fenton, 1997; Norberg, 1985; Padian, 1987; Scholey, 1986; Speakman, 2001), the ability to hang by the toes may have also been an important precursor to the evolution of flight. Freed from a role in compressive weight support during roosting, forelimb digits 2 to 5 could afford to become elongated to support the wing membranes (Norberg, 1985). Of all gliding mammals, only the ancestors to the bats subdivided the main gliding membrane with bony elements, and this subdivision has been suggested to have preadapted bats for flight by enabling them to perform the differential cambering, tensioning, and folding over the wing surface necessary for flapping flight (Hill and Smith, 1984; Speakman, 2001).

Kinematics of quadrupedal locomotion

The way in which quadrupedal mammals contact the ground with their forelimbs varies among taxa. Bears walk on the palms (carpal bones) of their outspread hands, dogs and cats walk on the proximal parts of the fingers (metacarpals), and ungulates walk on the tips of their fingers (phalanges). These are called plantigrade, digitigrade, and unguligrade stances, respectively, and the stance of almost any terrestrial mammal can be assigned to one of these forms (Lovegrove, 2004). However, because the fingers of bats are so long and slender, none of these stance types is

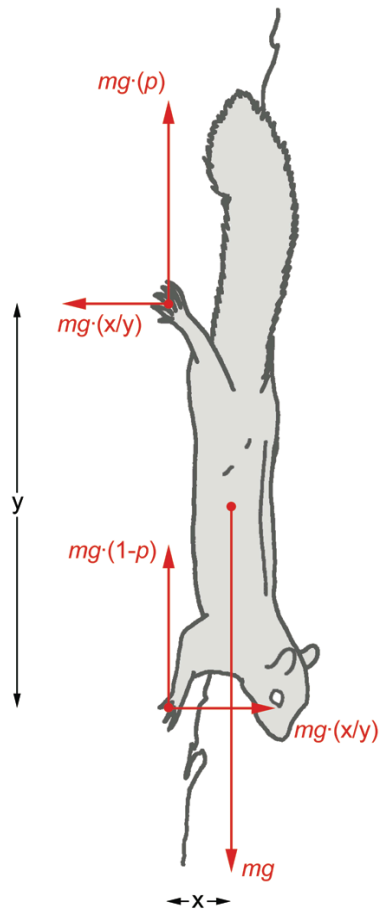


Figure 2: Hindlimb reversal is a common adaptation to arboreal niches among mammals, suggesting that it evolved in the arboreal ancestor to bats, prior to the evolution of flight. Note that in order to cling head-down to a vertical tree trunk, an animal must pull itself toward the surface with the hindlimbs: The gravitational force, of magnitude mg (where m is mass and g is the gravitational constant), pulls down on the animal's centre of mass, and is opposed by vertical forces applied at the forelimbs and hindlimbs, where the hindlimbs support any arbitrary proportion (p) of body weight. Because the gravitational and normal forces are separated by some distance (x), a torque acts about the hindlimb of magnitude $mg \cdot (x)$. This is opposed by the forelimbs, which press against the surface with a force of magnitude $mg \cdot (x/y)$, where y is the vertical distance between forelimb and hindlimb attachments. Horizontal forces can only be balanced by the attachment of the hindlimbs to the surface with a force of $mg \cdot (x/y)$ (based on Alexander, 2003).

available to them. Instead, the most terrestrial of bats close the wings and align the proximal digits with the forearms, so that only the ventral carpus and pollex (wrist and thumb) make contact with the ground. The hindlimbs are not so drastically modified as the forelimbs, and take a more 'reptile-like,' plantigrade sprawling position. When bats such as *D. rotundus* and *M. tuberculata* walk, the femora extend laterally and the soles of the hind feet contact the ground (Riskin et al., in press).

An important difference between terrestrially agile vampires and bats that walk awkwardly is that the vampires hold the abdomen above the ground at all times, whereas the bodies of other bats collapse periodically during shuffling (Lawrence, 1969; Riskin et al., 2005). With four limbs making contact with the ground, requirements for stability in bats are no different from those of any other quadrupedal animals (Cartmill et al., 2002). As a result, those bats that walk well use a lateral-sequence symmetrical walking gait similar to that used by many other tetrapods. The left hind foot moves forward in synchrony with the right forelimb, and vice versa, and as walking bats increase speed, they increase their stride frequencies just like terrestrial mammals do (Riskin and Hermanson, 2005; Riskin et al., in press).

Many bats possess the ability to hop, or 'leap-frog' (Lawrence, 1969) by pressing against the ground with the two forelimbs simultaneously. This results in a brief aerial phase before the bat lands some distance ahead of its original position. In some bats this may be necessary to the initiation of flight, permitting bats to attain a speed above stall-speed, or enough vertical clearance to accomplish complete wing strokes, but these hypotheses have

not been tested. In other species, including several that glean insect prey from the ground, like *A. pallidus* (Vespertilionidae), bats are able to launch directly into flight with a single jump. This behaviour is perhaps most advanced in Common Vampire Bats (*D. rotundus*), that, when jumping, apply a force equivalent to 9.5 body weights, to reach a take-off velocity of $2.38 \text{ m}\cdot\text{s}^{-1}$ in under 0.03 s (Schutt et al., 1997).

Desmodus rotundus are the only bats known to possess a true running gait, as distinguished by the presence of a notable aerial phase. Although peak speeds on a treadmill only reached $1.14 \text{ m}\cdot\text{s}^{-1}$ (Riskin and Hermanson, 2005), they can probably run as fast as $2.0 \text{ m}\cdot\text{s}^{-1}$ (Altenbach, 1979). The vampire bat running gait is kinematically different from that of any other tetrapod, chiefly because it is forelimb-driven. This gait appears to have evolved independently within the vampire lineage, probably from the wing-powered jumping behaviour mentioned above. There are no other examples in nature of a tetrapod lineage that has lost the ability to run only to acquire it afresh some time later. The independent evolution of a running gait by *D. rotundus* provides a novel group upon which to test hypotheses regarding cursorial locomotion, enabling researchers to better isolate the roles of biomechanical and phylogenetic constraints on the way in which gaits evolve.

How does anatomy influence crawling ability?

Many terrestrially agile bats possess anatomical specializations for walking. For example, to prevent the tips of the wings from dragging on the ground, *Cheiromeles* spp. and *M. tuberculata* tuck them into folds or

pouches of the wing (Dwyer, 1962; Schutt and Simmons, 2001). Also, whereas the membrane between the legs (uropatagium) of some bats is dragged on the ground during walking (Lawrence, 1969), in bats that walk well it is lifted either by a bony spur of the heel called a calcar (*M. tuberculata*; Dwyer, 1962), or by the tail (Molossids; Vaughan, 1959). However, the ability to lift the uropatagium may also have importance when bats feed or drink at the surface of water, so the utility of this specialization to terrestrial locomotion may only be a secondary consequence of adaptations for flight.

In Hairy-legged Vampire Bats (*D. ecaudata*), the calcar is free of the uropatagium, and assists in arboreal locomotion by acting as an opposable sixth digit, giving animals means to grip cylindrical branches (Schutt and Altenbach, 1997). A similar role is probably played by the opposable hindlimb digit I (hallux) of *Cheiromeles* spp. (Schutt and Simmons, 2001). It is relatively easy to see how these specializations assist in the terrestrial locomotion of ably walking bats. However, it is far more difficult to explain the anatomical bases of poor crawling. It would seem reasonable that interspecific variation in terrestrial agility should be reflected by differences in anatomy, since biomechanically relevant differences in ecology are often revealed by morphological variation (e.g. Dumont, 1997; Freeman, 1984; Norberg and Rayner, 1987). Quadrupedal locomotion has been considered in several studies of chiropteran anatomy (Altenbach, 1979; Howell and Pylka, 1977; Schutt and Altenbach, 1997; Schutt and Simmons, 2001; Strickler, 1978; Vaughan, 1959, 1970), but a clear morphological predictor of walking ability has been elusive. All that can be said with any certainty is that both the forelimb musculature and the hindlimb skeleton appear to be

important.

Several muscles of the forelimb skeleton are larger in species that walk well than in other species, including the *m. pectoralis abdominis*, *m. subscapularis*, *m. supraspinatus*, *m. rhomboideus*, and the *m. triceps brachii* (Strickler, 1978). This suggests that muscle proportions in the chest and shoulders influence terrestrial mobility, but the kinds of muscle tissue present may also be of importance. For instance, whereas the pectoralis muscles of all other bats surveyed possess only fast (type II) muscle fibres, those of vampire bats also include slow (type I) fibres (Hermanson et al., 1993; Hermanson et al., 1998). Maintaining the upright posture demonstrated by bats that are agile while walking might require slow, fatigue-resistant muscle, and the inability of most bats to hold themselves upright might be related to the absence of type I fibres. The musculature of other terrestrially agile bats, like *M. tuberculata* and *Cheiromeles* spp. has not been surveyed, but the presence or absence of type I fibres in those species will help clarify the importance of this character.

One of the most frequently cited explanations for differences in crawling ability is the hindlimb-strength hypothesis of Howell and Pylka (1977). They suggested that the bats that walk poorly do so because their slender legs are built for hanging, and that the femora of bats are too gracile to withstand the compressive forces associated with terrestrial locomotion. As evidence, Howell and Pylka pointed out that the legs of most bats are long and slender compared with the legs of terrestrial mammals of similar size, but that vampire bats have much thicker hindlimbs, comparable in diameter to those of terrestrial mammals (Fig. 3).



Figure 3: Femora of a terrestrially agile bat (*Desmodus rotundus*; a) and a bat that is not specialized for terrestrial locomotion (*Rhinolophus clivosus*; b). Bats of these species have similar body mass ranges, of around 20 to 30 g. Although it was once thought that poorly crawling bats were unable to walk because their femora were too fragile to resist the stresses associated with walking, bats that shuffle awkwardly actually place more weight on their hindlimbs while crawling than good walkers do. Thus the robustness of the femur does not prevent bats from walking well. Photograph by M. Brock Fenton.

To test the hindlimb-strength hypothesis, Riskin et al. (2005) compared the hindlimb ground-reaction forces of bats that walk well to those of bats that shuffle awkwardly. While the hindlimb-strength hypothesis predicted larger forces from the hindlimbs of ably walking bats, it was the poorly walking bats that actually placed the most weight on their legs. Based on these results, and on an engineering model-based reevaluation of the Howell and Pylka study, the hindlimb-strength hypothesis was rejected (Riskin et al., 2005). Thin legs do not prevent poorly crawling bats from walking well.

The influence of hindlimb morphology on terrestrial agility is probably related to femur orientation and mobility (Vaughan, 1959, 1970). The femora of those species that are totally unable to crawl extend caudally, whereas those bats that walk well extend the femora laterally to attain a sprawling reptile-like hindlimb posture. Crawling is made possible by specializations of the hip joint that enable the bat to swing its sprawling legs while walking, and thus through a range of motion that increase step length: the head of the femur is offset from the long axis of the bone to a greater degree in terrestrial bats than in poorly crawling species (Vaughan, 1970), and the acetabulum, with which the femur articulates, is also more wide in terrestrial bats, presumably allowing a greater range of motion (Dwyer, 1960b, 1962, 1970). Hindlimb mobility offers an intuitive mechanism to explain differences in crawling ability between able walkers and awkward shufflers, but it does not explain why some poorly crawling bats are able to shuffle awkwardly, dragging their limbs behind, whereas other bats are unable to walk at all.

Evolution of capable terrestrial locomotion

Dwyer (1960b) called *M. tuberculata* “the most sure-footed of all bats,” and Altenbach (1979) commented that “no other species possess the extreme terrestrial agility (of *D. rotundus*).” Both possess remarkable terrestrial agility, but the phylogenetic relationships of vampires and mystacinids strongly suggests that they evolved their quadrupedal abilities independently (Teeling et al., 2005). It appears that, whereas the vampires evolved terrestrial agility as a means of exploiting a relatively dangerous food resource, the short-tailed bats of New Zealand invaded the ground because there simply weren’t as many dangers there as faced by bats on the ground in other ecosystems.

Vampires possess walking agility as a specialization for their diet, the blood of mammals and birds (Greenhall, 1988). They belong to a neotropical family characterized by plant-feeding bats (Phyllostomidae), within a superfamily (Noctilionoidea) otherwise occupied mostly by insectivorous species. The three vampire species form a monophyletic outgroup (Desmodontinae) to all other members of their family (Jones et al., 2002), and they probably transitioned to blood-feeding from insectivory, not from frugivory (for a review of hypotheses concerning this transition, see Schutt, 1998). Being terrestrially agile is necessary for these animals, in part so that they are able to approach their prey stealthily by crawling on the ground or along a branch (Greenhall, 1988), but also to avoid injury or predation on the ground, since the neotropical distribution of the vampires overlaps with those of several bat predators.

Mystacinids also belong to the Noctilionoidea (Jones et al., 2002) and, like vampire bats, probably descended from an aerial insectivore

(Lloyd, 2001). However, the selective pressures that brought about terrestrial agility in mystacinids were almost certainly different from those of blood-feeding vampire bats. *M. tuberculata* is restricted to New Zealand, where it is one of only two endemic non-marine mammals (the other is a distantly related vespertilionid bat, *Chalinolobus tuberculatus*). In the absence of terrestrial mammals as competitors or predators, *M. tuberculata* adopted a shrew-like niche, foraging in leaf litter for invertebrates (Jones et al., 2003). *M. tuberculata* also feed on fruit and pollen (Daniel, 1976), and take a significant proportion of their prey on the wing, in the manner of typical insectivorous bats (Arkins, 1996).

M. tuberculata previously shared their terrestrial niche in New Zealand with a sister species, *M. robusta*, that also foraged terrestrially (Hill and Daniel, 1985). However, that species went extinct around 1967, after the introduction of rats to their range. Populations of *M. tuberculata* have disappeared from several rat-infested islands and overall have suffered declines so severe that the species has been placed in the category of highest conservation priority by the New Zealand Department of Conservation (Molloy, 1995).

It is difficult to understand the evolutionary progression that led to capable crawling in some bats because we do not completely understand the biomechanical basis of differential crawling abilities among bats generally. Our understanding is limited by the fact that the basic kinematics are yet to be recorded for almost every species. First priority should be given to those species that walk well (*M. tuberculata*, *Cheiromeles* spp., *T. australis*, *A. pallidus*, *M. septentrionalis*, etc.), but data on the awkward shuffling of other bats will be helpful as well. With a range in body sizes

spanning nearly three orders of magnitude, and a staggering diversity of physiological and anatomical specializations (Simmons, 2005), the aerial and non-aerial locomotion of bats could provide a convenient model system for the study of biomechanical tradeoffs.

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CHAPTER 2: TESTING THE HINDLIMB-STRENGTH HYPOTHESIS: NON-AERIAL LOCOMOTION BY CHIROPTERA IS NOT CONSTRAINED BY THE DIMENSIONS OF THE FEMUR OR TIBIA*

Summary

In the evolution of flight bats appear to have suffered a tradeoff; they have become poor crawlers relative to terrestrial mammals. Capable walking does occur in a few disparate taxa, including the vampire bats, but the vast majority of bats are able only to shuffle awkwardly along the ground, and the morphological bases of differences in crawling ability are not currently understood. One widely cited hypothesis suggests that the femora of most bats are too weak to withstand the compressive forces that occur during terrestrial locomotion, and that the vampire bats can walk because they possess more robust hindlimb skeletons. We tested a prediction of the hindlimb-strength hypothesis -- that during locomotion, the forces produced by the hindlimbs of vampire bats should be larger than those produced by the legs of poorly crawling bats. Using force plates we compared the

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hindlimb forces produced by two species of vampire bats which walk well (*Desmodus rotundus* N=8 and *Diaemus youngi* N=2) to the hindlimb forces produced during over-ground shuffling by a similarly sized bat which is a poor walker (*Pteronotus parnellii* N=6). Peak hindlimb forces produced by *P. parnellii* were larger (ANOVA; $P < 0.05$; $n=65$) and more variable (mean 93.5% body weight \pm S.D. 36.6%) than those of *D. rotundus* ($69.3 \pm 8.1\%$) or *D. youngi* ($75.0 \pm 6.2\%$). Interestingly, the vertical components of peak force were equivalent among species ($P > 0.6$), indicating similar roles for support of body weight by the hindlimbs in the three species.

We also used a simple engineering model of bending stress to evaluate the support capabilities of the hindlimb skeleton from the dimensions of 113 museum specimens in 50 species. We found that the hindlimb bones of vampires are not built to withstand larger forces than those of species that crawl poorly. Our results show that the legs of poorly crawling bats should be able to withstand the forces produced during coordinated crawling of the type used by the agile vampires, and this indicates that some mechanism other than hindlimb bone thickness, such as myology of the pectoral girdle, limits the ability of most bats to crawl.

Introduction

When opposing selective pressures have acted on an anatomical trait the result can be an evolutionary trade-off, whereby the current ability of an organism to perform one task is hindered by requirements for some other aspect of its life history (Vanhooydonck and Van Damme, 2001). This appears to have been the case in the evolution of locomotion by the bats

(Mammalia: Chiroptera). All bats possess capable powered flight, but most are poor terrestrial locomotors relative to ground-dwelling mammals of similar size (Lawrence, 1969).

Compared to similarly sized terrestrial mammals, the bones of a bat's forearm are long and curved, the radius is large relative to the ulna (with which it is often fused), and the digits are elongated as a supporting frame for the membranous wings. The hindlimb skeleton is also extensively modified: The fibula is reduced or absent, the femur and tibia are long and slim relative to those of similarly sized terrestrial mammals, and these bones are rotated 90 to 180° from the typical mammalian pattern. As a result, the femora extend laterally or caudally, and the flexor surfaces of the knees face ventrally. This combination of specializations is presumed to adapt bats to flight (Simmons and Geisler, 1998; Strickler, 1978; Swartz et al., 1992; Vaughan, 1959). They are not seen together in any of the terrestrial mammals, and most likely underlie the general trend of poor walking ability seen in bats.

While the vast majority of the >1,100 species of bats crawl poorly, coordinated terrestrial locomotion does occur in a few phylogenetically disparate bat species (Teeling et al., 2003; Teeling et al., 2002). Several molossid bats walk well (Dietz, 1973; Strickler, 1978), most notably *Cheiromeles* spp. These animals possess distinctive subaxillary pouches where the tips of the folded wings are held during walking (Schutt and Simmons, 2001). In addition, the Short-tailed Bats (Mystacinidae: *Mystacina tuberculata*) forage terrestrially and even burrow (Daniel, 1979), having invaded a terrestrial niche in New Zealand that is more typically occupied by insectivoran mammals elsewhere. The most studied of the walking bats are

the vampires (Phyllostomidae: *Desmodus rotundus*, *Diaemus youngi*, *Diphylla ecaudata*). These bats constitute a monophyletic group of obligate blood-feeders (Baker et al., 1989). All three species are known to approach their prey by walking over a substrate, either over ground or along the surface of a branch (Greenhall and Schmidt, 1988).

It is not clear whether the walking ability of different bat species can be predicted by any morphological differences among them. Strickler (1978) observed that in bats which walk well, several muscles of the shoulder (m. pectoralis abdominis, m. subscapularis, m. supraspinatus, m. triceps brachii, and m. rhomboideus) are enlarged, and suggested distinct roles for those muscles during crawling. However, he did not provide a predictive model of crawling ability based on muscle dimensions. A more numerical approach was taken by Howell and Pylka (1977), who observed that the ratio of femur length to diameter in bats and found that the allometry of this ratio differs from the typical mammalian pattern; the femora of bats are longer and more gracile than those of terrestrial mammals. They hypothesized that this morphological difference meant that the legs of bats could not support the body's weight during crawling. Howell and Pylka noted that the femora of vampire bats were more robust than those of other bats, and suggested that the improved walking ability of vampires was due to their improved ability to support weight with the legs.

The Howell and Pylka study has been cited widely in the popular (Why bats hang upside down: *Omni*, vol. 1(2), p. 38, 1978) and scientific literatures (Jungers, 1979, 1984; Norberg, 1981; Schutt, 1993; Simmons and Geisler, 1998; Smith et al., 1995; Swartz, 1997; Swartz et al., 2003), but the hindlimb-strength hypothesis has not yet been experimentally tested. We do

this by directly measuring the forces produced by the hindlimbs of walking vampire and non-vampire bats.

The hindlimb-strength hypothesis has two components: that the skeletons of most bats are too weak to withstand the ground reaction forces associated with terrestrial locomotion, and that the vampire bats walk well because their hindlimbs are stronger than those of other bats. If these components of the hypothesis are both correct, the legs of vampires are predicted to withstand forces during walking that the legs of other bats cannot. Therefore the hindlimb ground reaction forces produced during terrestrial locomotion by vampire bats will be larger in magnitude than those of poorly crawling species. If the forces transmitted by the hindlimbs of poorly crawling bats are as large as those of vampires, the hindlimb-strength hypothesis would be rejected. However even then, robustness could reflect some other capacity, such as manoeuvrability or speed, which lends vampires their improved terrestrial ability over other bats. We examine the dimensions of femora and tibiae in a broad range of bat species, to verify that the limbs of vampires are more robust than those of other bats, and comment on how the allometric relationships among external limb dimensions might relate to function in the bats.

Materials and methods

Force platform and video analysis

Study animals

To represent bats with the ability to walk terrestrially we chose two species of vampire bats, *Desmodus rotundus* Wied, 1826 (N=8) and *Diaemus youngi* (Jentink, 1893) (N=2). These were compared to a poorly crawling insectivorous bat of similar size, *Pteronotus parnellii* (Gray, 1843) (Mormoopidae; N=6). We also made behavioural observations of *Natalus tumidirostris* Miller, 1900 (Natalidae; N=5), which are not known to crawl. The subject animals were caught using mist nets at various locations in Trinidad, West Indies during August 2003 and July 2004. Some animals were also collected directly from their roosts with hand nets. In all cases, locomotion studies were conducted within 24 h of capture. All animals were handled in accordance with permits issued by the Ministry of Agriculture (Forestry Division) of Trinidad and Tobago, and protocols were approved by the Institutional Animal Care and Use Committee at Cornell University.

Platform construction

Following improvements on Heglund's (1981) original design by Biewener and Full (1992), we constructed two force-sensitive platforms, serially set in a runway, to measure the ground reaction forces of the hindlimbs as animals walked or crawled sequentially across their surfaces. We designed and built the platforms to be highly sensitive, but also so that they could be easily transported to field locations. In further reference to

these measurements, the axis parallel to the direction of travel is denoted as x , the orthogonal horizontal axis as y , and the vertical axis as z .

Each of our platforms consisted of a 74.6 mm (x) by 155.0 mm (y) honeycomb fiberfoam plate, supported at either end by two hollow aluminum box beams oriented parallel to the y axis. These beams rested at their ends on short box beams glued to a heavy aluminum base plate. We used Trubond Clear 2-ton Epoxy (Devcon, Danvers MD, USA) to attach the fiberfoam plate to the beams, and specialized epoxy (J-B Weld, Sulphur Springs TX, USA) for all aluminum-aluminum joints. At certain sites the aluminum box beams were milled to form a series of double cantilevers (Biewener and Full, 1992), each oriented so that they were perpendicular to one of the three orthogonal axes. A force applied to the surface of a plate caused bending in the cantilevers, which was measured via strain gauges bonded to them (Micromasurements Corp., Raleigh NC, USA).

The strain gauges were wired into four 3.3 V Wheatstone bridge circuits. Each bridge input and output was connected to one channel of a multi-channel strain-conditioning isolation amplifier (National Instruments Corporation, Austin TX, USA; SCXI 1000 chassis containing two SCXI 1121 modules with SCXI 1327 terminal blocks). The analog data were digitized (National Instruments DAQCard-1200) and saved to a laptop computer (Apple Macintosh PowerBook) running a custom-made acquisition program (LabVIEW 6.1). Forces in the z -direction were measured separately at the front and rear supporting beams of each plate so that the position of the centre of pressure along the x -axis could be determined from the relative output of the two channels (Heglund, 1981). Horizontal channels were

monitored with one output each because horizontal forces can only be applied at the surface of the plate.

Platform performance verification and calibration

The functional capabilities of the platforms were evaluated on the basis of resonant frequency response and repeatability of load response (calibration). The former determines the minimum reliable response time of the plate and indicates the loading-rate limit at which useful data can be observed using the instrument.

We measured the resonant frequency of each axis by applying a sharp blow to the plate surface with the tip of a pen, and measuring the rate of oscillation after contact (Biewener and Full, 1992). One platform had a resonant frequency at 457 Hz (x), 128 Hz (y), 458 Hz (z), and the other at 480 Hz (x) 156 Hz (y) 480 Hz (z). Using the lowest of these values, the platforms allowed reliable event records on the order of 7.8 ms.

Both platforms were calibrated on each day that measurements were taken, using the methodology described by Biewener and Full (1992). Briefly, horizontal location of force along the x-axis was determined by placing a 100 g mass at a series of different locations on a force plate. From the relative difference in output between the front and rear vertical circuits, the voltage output could be related to the known positions of force application. Force magnitude-voltage relationships of each channel were determined using a series of known loads calibrated against the voltage output in each direction. For this calibration the front and rear z-oriented channels were summed to represent total vertical load. Regressions of force to voltage were linear on all channels, with $r^2 > 0.999$. Electronic drift in the

baseline output was determined separately for each individual trial by sampling the signals from each channel of an unloaded plate (zero force) within 10 s of data collection.

Because our platforms were designed to measure relatively small forces, they were also susceptible to noise generated by small vibrations in the environment and stray electrical interference. These artifacts were removed through digital filters; a Butterworth band-stop of 58-62 Hz eliminated AC-generated noise, and a 100 Hz Butterworth low-pass filter eliminated all higher-frequency noise.

Force records were successfully collected from all three force plate axes in the 2004 field season. Calibration problems for the horizontal axes made these records unreliable in 2003, so only vertical forces from that field season were included in our analyses.

Video recordings and synchronisation with force measurements

A Plexiglas cage, 0.48 (x) by 0.15 (y) by 0.11 (z) m, was used to contain the animals while we observed their locomotion. The force plates comprised the centre of the cage floor. We placed a MotionMeter 250 digital high-speed camera (Redlake Systems, San Diego CA, USA) ca. 2 m from the cage, level with the surface of the plate. A mirror above the cage that was tilted 45° from horizontal permitted simultaneous views of the plates from the side (y) and above (z).

A square-wave signal from the master/slave port of the video camera was sent to both an LED next to the plate in the camera view, and to the laptop (via the SCXI strain gauge amplifier). In each trial the signal was interrupted briefly by means of a hand-held switch. This event was clearly

visible on the computer files as a change in the shape of the square wave, and on the video recordings as the interruption of the LED emission. These signals were used to synchronize the video sequences with force-plate output, to a resolution of 4 ms.

Trials and Analyses

To record the forces produced by the hindlimbs during locomotion, an individual bat was placed at one end of the Plexiglas enclosure. We encouraged it to walk across the force plates by blowing on it through a straw. As the animal crossed the force plates, video (250 Hz) and force plate data (1000 Hz) were recorded simultaneously.

From each trial where a bat moved at a relatively steady speed across the force plate, we isolated the span of time where only the hindlimbs were in contact with a plate. The first and last 10 ms of the selected interval were eliminated to account for the time resolution of our force plate outputs. From each trial we recorded the magnitude and direction of the peak ground-reaction force, calculated as the vector sum of forces in the x , y , and z directions. Jumps and stationary standing were omitted from analyses.

We measured the total force experienced by the hindlimb skeleton in every trial, regardless of how many feet were in contact with the ground. In all three species tested, several of the peak hindlimb forces occurred when only one of the hindlimbs was in contact with the force plate, while others occurred while both feet were in contact. Our methods did not permit us to determine the relative contributions of two feet in simultaneous contact with a single force plate.

In order to understand how the limb bones of the poorly crawling bat, *P. parnellii*, were loaded during locomotion, we recorded the angle θ between the net ground reaction force vector and the long axis of the tibia. Since the force contributions of each leg could not be isolated in most trials, this analysis was restricted to those trials in which peak force occurred as a single limb contacted the plate. We were unable to perform similar measurements for the femur, as there were too few trials in which its orientation could be clearly discerned.

Museum specimens

Hindlimb measurements

We measured the greatest lengths and least diameters (to 0.1 mm) of right femora and tibiae of 113 museum specimens spanning 50 species in 12 of the 17 currently recognized chiropteran families (Teeling et al., 2002). We examined specimens from as many families as possible from the museums we visited and did not choose our specimens with regard to any criteria other than availability. We obtained body-mass estimates for each species from the literature. Where only a body-mass range was available, we took the midpoint of the range as our estimate. Our sample ranged in body mass across three orders of magnitude, and approximates an unbiased sample of chiropteran hindlimb diversity.

Both internal and external dimensions will influence the stress developed within a long bone due to an applied bending load. When evaluating the structural capacity of long bones based primarily on external dimensions it is important to verify the underlying assumption that relative cortical thicknesses remain consistent between groups compared. We were

unable to make direct measurements of cortical thickness for all species included in the dimensional analysis. In order to evaluate the potential for differences between cortical dimensions of terrestrially active and non-ambulatory species we compared the cortical thickness of femora and tibiae of *D. rotundus* and a non-vampire bat species, *Myotis lucifugus* (Vespertilionidae). Measurements were taken from radiographs of five right hindlimb skeletons of each species in mediolateral and dorsoventral views. The percentage of a bone's diameter that was occupied by cortex in each of the two views was averaged, and these measurements were compared between species.

Comparison of vampire bats with non-vampire bats

We applied the external femur and tibia dimensions of bats to two models. First, we repeated the procedures of Howell and Pylka (1977), using least-squares regressions of log-log plots to compare the allometric relationship of length to diameter found in the femora and tibiae of vampire and non-vampire bats. Since ordinary least squares regression is no longer generally considered an appropriate tool for studies of allometry (LaBarbera, 1989), we also applied reduced major axis regressions (RMA) to the same data. Second, we applied the same limb dimensions to an engineering-based bending model of bone stress. If the bones of vampire bats really are built to withstand the forces of walking better than those of other bats, they should be subject to smaller stresses during walking than those of other bats.

For simplicity, we modeled each bone as a cylinder of uniform diameter δ and length λ . When a force F is applied at some angle to the end

of a cylinder, it can be separated into components parallel and perpendicular to the cylinder's long axis. The relative magnitude of each depends on the angle θ between the force vector and the long axis of the cylinder. The total stress (σ) can be calculated as follows (Gere, 2001):

$$\sigma_{estimated} = \frac{4F}{\pi \cdot \delta^2} \cdot \left(\cos \theta + \frac{8 \sin \theta \cdot \lambda}{\delta} \right) \quad (1)$$

Because stress is unevenly distributed across the diameter of a cylinder when it is loaded in bending, stresses imposed by bending will greatly exceed those from compression. This is especially true of long, thin cylinders. Therefore, the greatest stresses for the femora and tibiae of bats are generated when a force acts perpendicular to the long axis of the bone ($\theta = 90^\circ$). In this case, the equation simplifies to a single term:

$$\sigma_{estimated} = \frac{32F \cdot \lambda}{\pi \cdot \delta^3} \quad (2)$$

If we assume that the forces applied to the hindlimbs scale with body mass (m_b) across species, we can obtain a relative estimate of bone stress as follows:

$$\sigma_{relative} \propto \frac{m_b \cdot \lambda}{\delta^3} \quad (3)$$

Relative stress does not provide an absolute estimate of the stresses endured by bat bones, but provides a means by which the strengths of bat limbs can be compared among species. Because the numerical values of relative stress are arbitrary, we assigned a value of 1.0 to the $\sigma_{relative}$ of the tibia in the more thin-legged of the two vampires in this study, *D. youngi*. If, as the hindlimb-strength hypothesis predicts, the legs of vampires are more

robustly built than those of other mammals, it follows that $\sigma_{relative}$ values of all non-vampire bats should be significantly greater than 1.0.

Our model assumes that the forces a bat exerts during terrestrial locomotion are proportional to its body mass, and that the stresses vary among species as a result of bone dimensions. Alternatively, it is possible that the stresses experienced by the hindlimbs of all bats are similar during terrestrial locomotion, and that the magnitudes of the forces vary according to bone dimensions. However this distinction is unimportant, as the two models have numerically equivalent predictions and conclusions.

Results

Force platform and video analysis

Kinematics of non-aerial locomotion

Pteronotus parnellii exhibited no consistent gait across trials (Fig. 4a). During crawling, limb movement patterns were highly variable, with kinematics similar to those described for several vespertilionid and phyllostomid bats (Dietz, 1973; Lawrence, 1969). Typically, the body rested in contact with the substrate, with the limbs in a sprawling position. To initiate forward motion the body was lifted by adduction of the forelimbs. The head and torso moved anteriorly 0.25 to 0.5 body lengths as the forearms rotated dorsoventrally and the legs shuffled forward. The bat then lifted its wings dorsally and the thoracic region collapsed to the ground. The forearms generally moved together, but their motions were not symmetrical, and animals frequently tilted or fell to one side during crawling.

Figure 4: Typical locomotory sequences for (a) *P. parnellii*, (b) *D. rotundus*, and (c) *D. youngi*. Images are at 44 ms intervals. In those images for which only the hindlimbs are in contact with the left plate, the normal force for that plate is shown as a yellow arrow. The graph below each image shows the magnitude of the force on the left plate over the course of the image sequence. Open yellow circles indicate the timing of images with force vectors. Solid circles give the times of all other frames. Note that the magnitude of the force vector for both vampire species decreases gradually as the animal shifts its weight forward, but that the forces are highly variable for the poorly crawling bat, *P. parnellii*.

Figure 4a

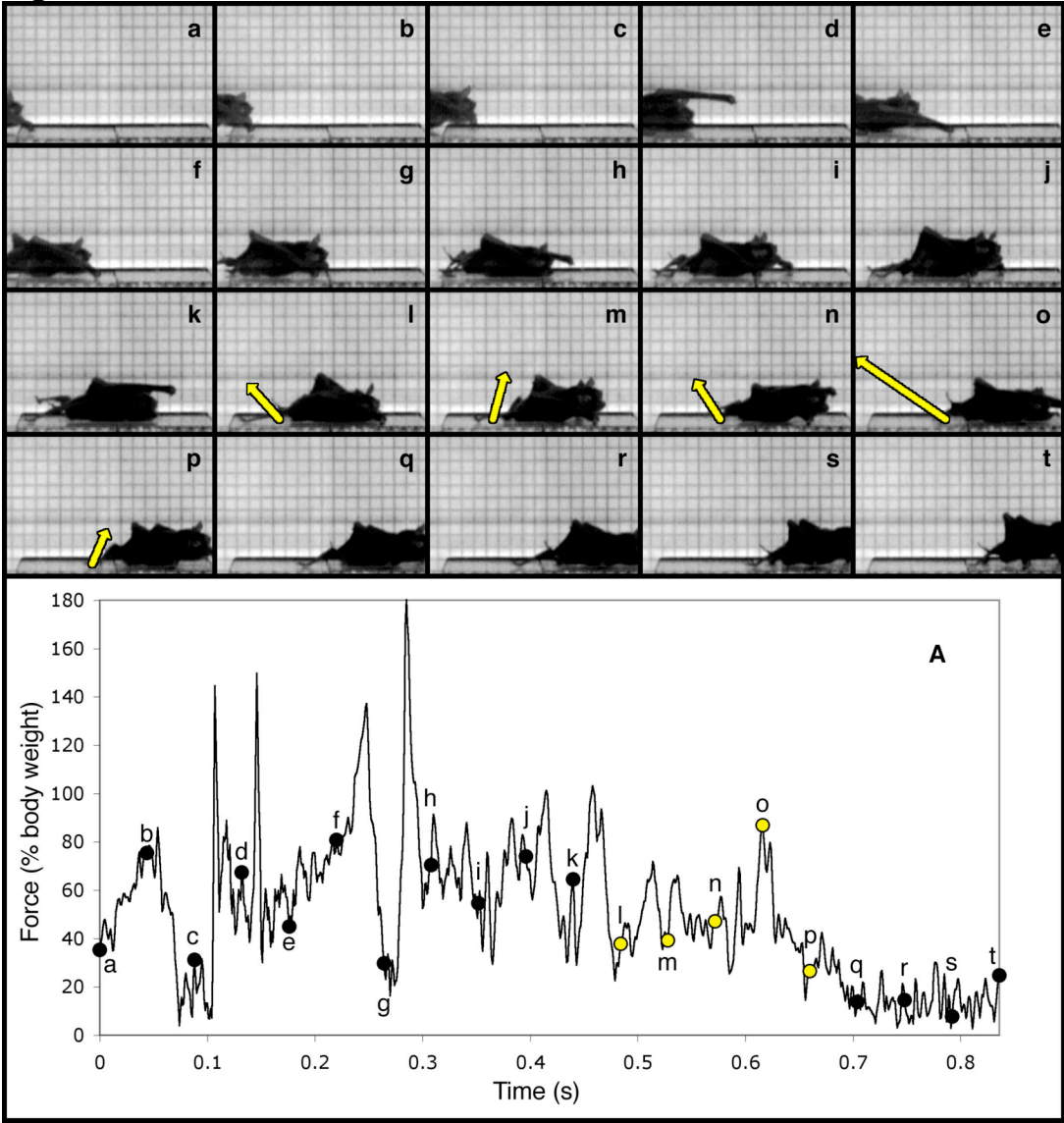


Figure 4b (continued)

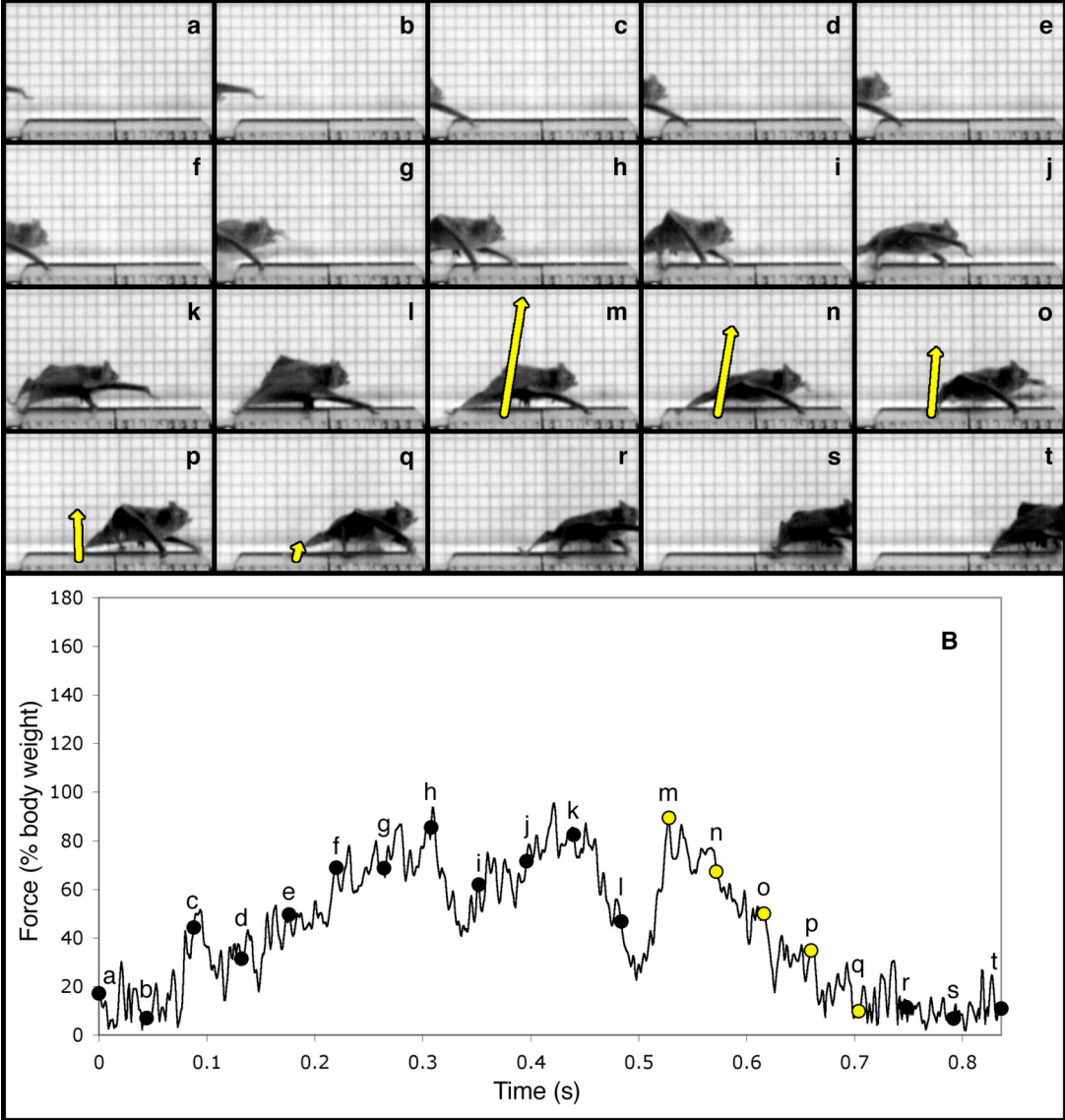
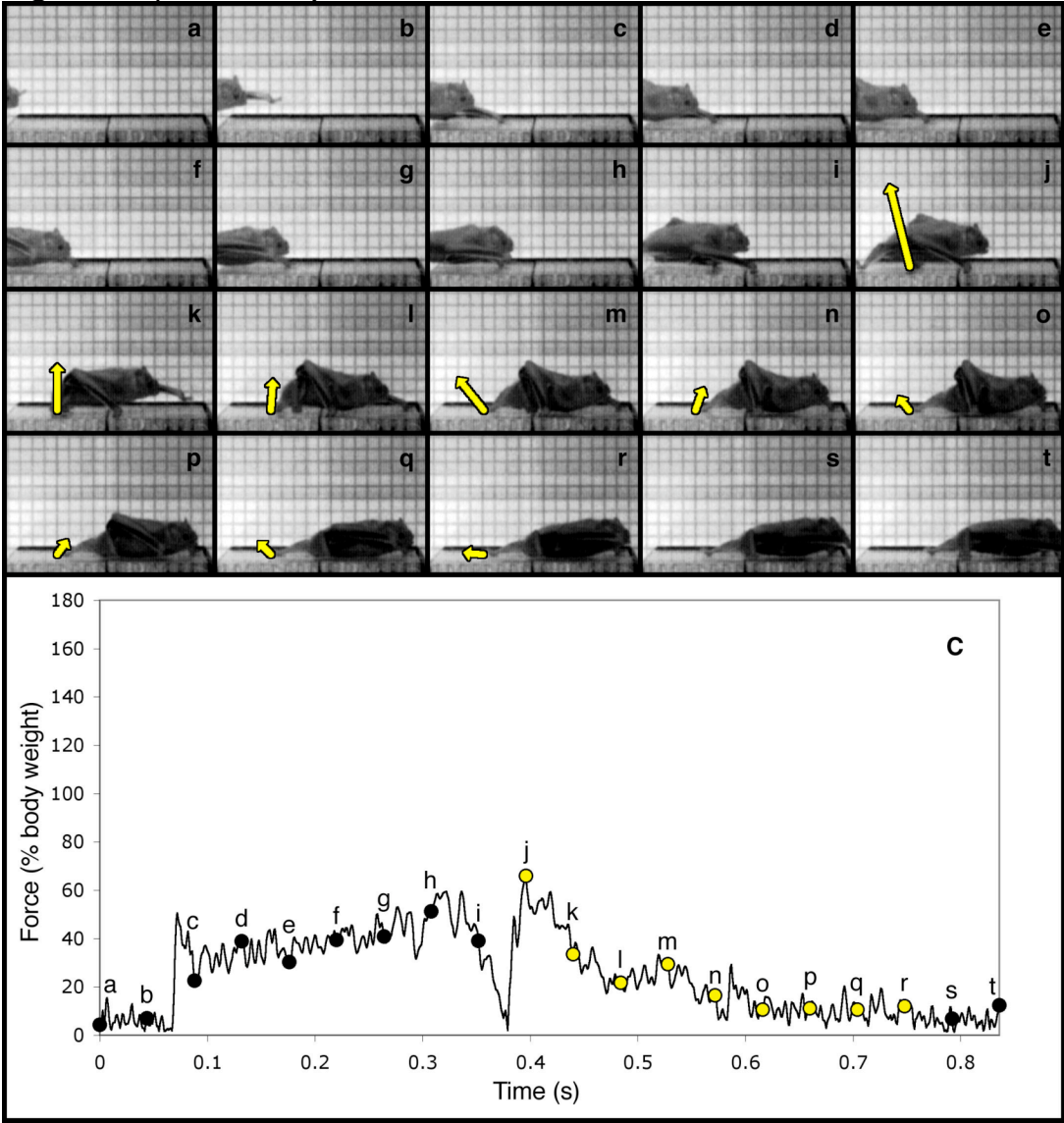


Figure 4c (continued)



During forward crawling, the femora were directed dorsolaterally and held roughly horizontal. The tibiae pointed caudally and occupied angles ranging from 5 to 40 degrees from horizontal. We did not observe contact between the floor and any part of the hindlimbs other than the pelvic girdle and the plantar surfaces of the feet. Peak hindlimb forces typically occurred while the torso was not in contact with the ground, suggesting that the hindlimbs played a role in supporting body weight.

We do not describe the gaits of *D. rotundus* and *D. youngi* in detail here because they did not differ from detailed descriptions available in the literature (Altenbach, 1979; Schutt et al., 1999). Both species used a lateral-sequence symmetrical walking gait (Hildebrand, 1985) in which only the plantar surfaces of the feet and the carpi and pollices of the forelimbs made contact with the substrate (Figs. 1b,c). Animals held their abdomens above the ground at all times. The ventral surface of the abdomens of *D. youngi* were ca. 1 cm from the floor and those of *D. rotundus* were ca. 2.5 cm. Peak hindlimb forces typically occurred just after a forearm was lifted from the plate. Ground reaction forces at the hindlimbs decreased as the bat placed its forelimb on the ground and shifted the centre of mass anteriorly. Forces declined to zero as the bat lifted its feet to take the next step.

We also introduced bats of a fourth species, *Natalus tumidirostris* to the enclosure, but none conducted crawling locomotion. Instead, individuals initiated flight by leaping vertically from the plate by means of strong downward thrusts of the wings, and flew to the end of the enclosure. We did not use the trials from this species in any of our analyses, but present them here as an example of a species that does not crawl.

Hindlimb forces

The body masses of bats in this study were similar, though *D. youngi* were slightly larger (27.0 g and 36.0 g; N=2) than *D. rotundus* (23.1 ± 2.4 g; N=8) or *P. parnellii* (19.1 ± 1.2 g, N=6). To account for differences in body size among individuals, we report all forces as a percentage of body weight.

Contrary to the predictions of the hindlimb-strength hypothesis, we found that at the time of peak hindlimb force production the legs of the poorly crawling insectivore, *P. parnellii*, were loaded with significantly larger forces (93.5% of body weight \pm 36.6%) (mean \pm s.d.) than those of *D. rotundus* (69.3% \pm 8.1%) or *D. youngi* (75.0% \pm 6.2%) (ANOVA with Tukey-Kramer; $n=65$; $P<0.05$). The magnitudes of maximum forces were also most variable in *P. parnellii* (Levene test; $n=65$; $P<0.0001$), reflecting the highly variable movements performed by that species (Fig. 5a).

The directions of peak hindlimb ground reaction forces were nearly vertical in *D. rotundus* ($73.6^\circ \pm 10.8^\circ$) and *D. youngi* ($75.5^\circ \pm 6.7^\circ$), while forces produced by *P. parnellii* ($61.7^\circ \pm 16.7^\circ$) were less vertically directed (Kruskal-Wallis; $n=65$; $P<0.01$). The vertical component of peak hindlimb force did not differ significantly among the three taxa studied (ANOVA; $n=84$; $P>0.6$), even though the maximum force applied by the hindlimbs was greater in *P. parnellii* (Fig. 5b). This occurred due to the larger horizontal force component of *P. parnellii*. The similar vertical force contribution likely indicates that the hindlimbs of all three species contributed equally to support of body weight against gravity.

In those *P. parnellii* trials in which a single hindlimb contacted the ground at peak force, we were able to measure the angle θ between the force vector and the long axis of the tibia. The sine of this angle, which is

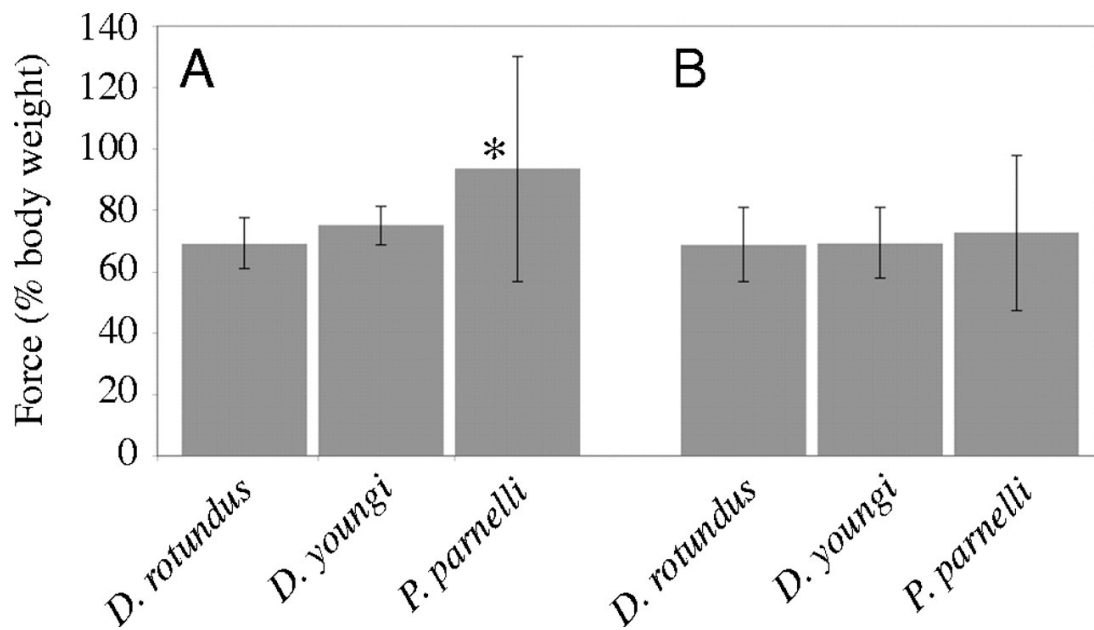


Figure 5: Magnitudes of hindlimb forces in *D. rotundus*, *D. youngi*, and *P. parnelli*: (A) total force, calculated as the vector sum of forces in the x, y, and z directions; (B) vertical component of peak force. Asterisk denotes significance at $P<0.05$.

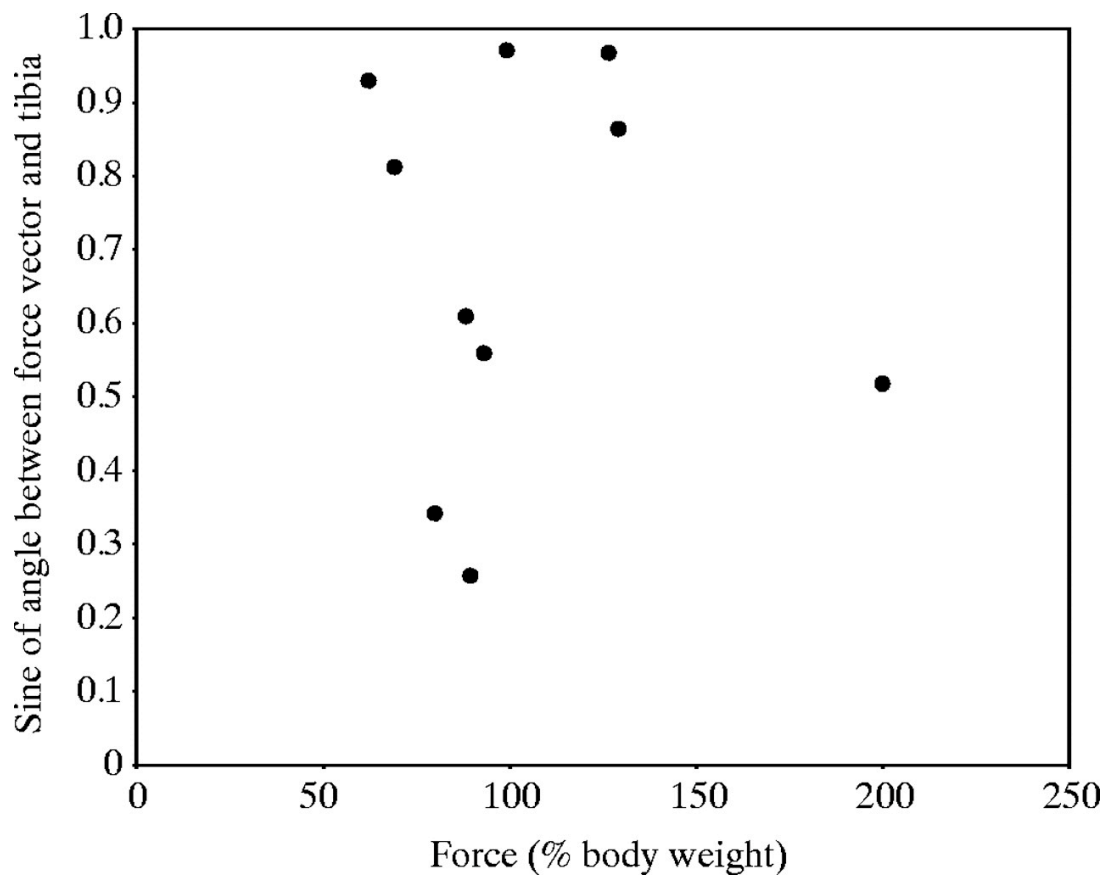


Figure 6: Sine of angle (θ) between force vectors and the long axis of a tibia vs. magnitude of peak force in *P. parnellii* trials, where peak force occurred as a single leg was in contact with the plate.

proportional to the bending stress of the tibia (Equation 1), was highly variable (0.68 ± 0.26 ; $n=10$). No correlation existed between the magnitude of the force and $\sin \theta$ (F-test, $n=10$; $P>0.9$; Fig. 6).

Museum specimens

Allometry of limb bones

Across species, femur length scaled to $M_b^{0.30}$ ($r^2 = 0.78$; $n = 50$) (RMA: $M_b^{0.38}$), while tibia length scaled to $M_b^{0.32}$ ($r^2 = 0.73$; $n = 49$) (RMA: $M_b^{0.43}$). The exponents of these least square regressions are comparable to values reported for femora (0.18 to 0.36) by Howell and Pylka (1977) and for tibiae (0.27 to 0.42) by Norberg (1981), suggesting that our sample of museum specimens was representative of the group and not biased by the availability of specimens for this study.

Our least squares regressions of length to diameter in the long bones of bat limbs also closely match those of Howell and Pylka (1977). Excluding vampire bats from the analyses, femur lengths of bats scaled to diameter^{0.78} ($r^2=0.81$; $n = 48$) (RMA: $M_b^{0.97}$), while tibia lengths scaled to diameter^{0.63} ($r^2=0.44$; $n = 45$) (RMA: $M_b^{1.43}$).

The lengths of vampire bat femora in our study were proportional to diameter^{0.18} and the lengths of tibiae were proportional to diameter^{0.21}. These results are similar to those of Howell and Pylka (1977). The r^2 values of our least squares regressions were 1.0, since they each consisted of only two species. We recognize that two data are clearly not sufficient for an allometric study (which is why we do not report the RMA regression values), but the Howell and Pylka (1977) study included only three data in the

vampire bat regression, and our purpose was to compare their results to our own.

Despite these differences of allometric function exponent between vampire and non-vampire bats, the hindlimb bones of vampire bats did not fall outside the least-squares 95% confidence interval of the length-diameter ratio prescribed by the other bats in this study (Fig. 7). In other words, the length to diameter ratio of vampire bats does not fall outside the range of variation which exists among non-vampire bats.

We found that cortical thickness was greater in *M. lucifugus* (N=5) than in *D. rotundus* (N=5), for both femora (t-test, $P < 0.001$, $n=10$) and tibiae (t-test, $P < 0.001$, $n=10$). Although the cortex was not uniform along the length of any bone, we found that in mid-point femoral cross-sections, cortex occupied 66.7% of radius $\pm 3.3\%$ in *M. lucifugus* and 41.3% $\pm 1.8\%$ in *D. rotundus*. For tibiae, cortical thickness was 71.0 $\pm 10.1\%$ in *M. lucifugus* and 35.3% $\pm 6.0\%$ in *D. rotundus*.

Estimation of relative bone stresses

There was a slight trend for $\sigma_{relative}$ to increase with $\log M_b$ for femora ($r^2=0.25$) and tibiae ($r^2=0.36$). Vampire bats did not possess more structurally stable hindlimbs than those of all other bats in our study (Fig. 8). The values of $\sigma_{relative}$ for *D. rotundus* and *D. youngi* femora were first and sixteenth lowest respectively among all species ($n=50$), while relative tibia stresses were fourth and eighteenth lowest respectively ($n=47$). The lowest $\sigma_{relative}$ we calculated among tibiae was that of *Molossus molossus* (Molossidae). The highest predicted bone stresses in our study were those

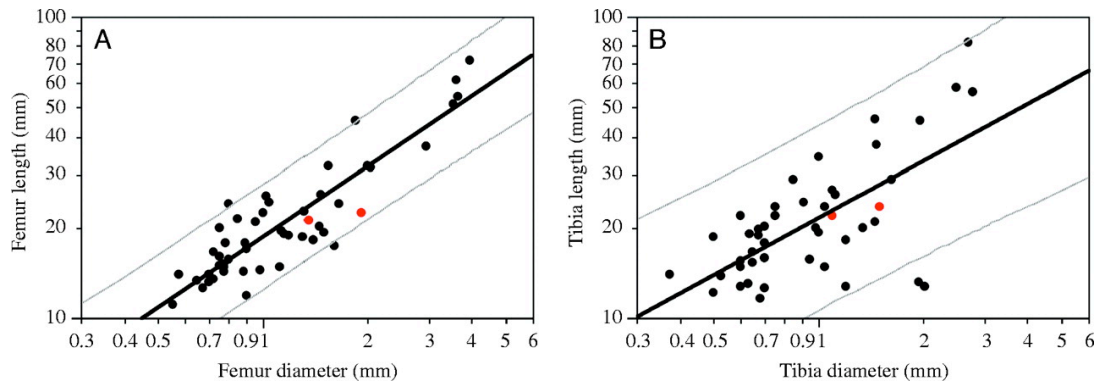


Figure 7: Log-log plots of length to diameter for bat (a) femora (b) and tibiae. Red circles denote vampire bats. Other bat species are black. Bold line represents best fit and grey lines indicate 95% confidence interval from least squares regression of non-vampire bat data only.

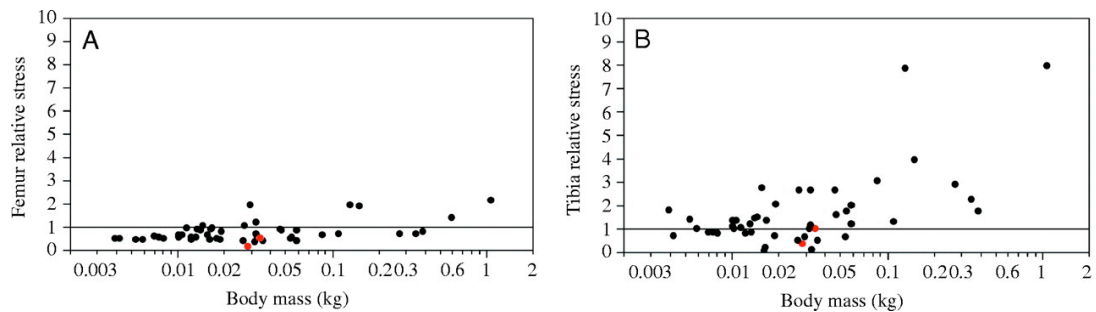


Figure 8: Relative hindlimb bone stresses for femora (a) and tibiae (b) across the range of body masses in this study. Red circles represent vampire bats (*D. rotundus*, *D. youngi*), black circles represent other species. According to the hindlimb-strength hypothesis, the non-vampires should have relative bone stress values greater than that of *D. youngi* tibiae (1.0).

of the tibiae of *Hipposideros commersoni*, a large-bodied (0.13 kg) predatory species, and the frugivore *Pteropus vampyrus*, the largest bat (1.08 kg) in our sample.

Discussion

Inference of hindlimb strength from external bone dimensions

The hindlimb-strength hypothesis rejected

The legs of *P. parnellii* were loaded with larger forces than those of vampires, but did not break. It is therefore clear that the bones of *P. parnellii* would be able to withstand the forces associated with coordinated non-aerial locomotion. However, *P. parnellii* are poor walkers relative to *D. rotundus* and *D. youngi*. This is reflected by their inability to hold the abdomen above the floor during crawling, and by the higher variation in magnitudes and directions of force vectors applied by the hindlimbs. These results demonstrate that some mechanism other than frailty of the hindlimb skeleton prevents *P. parnellii* from walking smoothly.

The hindlimb-strength hypothesis inferred differences in hindlimb strength from the allometric relationship between length and diameter in the femora of bats. This approach was flawed in two ways. First, although the exponents of the allometric relationships of vampire and non-vampire bats differ, the vampire bat data points fall within the non-vampire regression. The argument that vampire bats are built differently than other bats would only have structural consequences beyond the body sizes of the vampire bats. Second, the ratio of length to diameter in a bone does not necessarily

determine strength. A simple model of bone strength suggests that the leg bones of vampires are not significantly stronger than those of non-terrestrial bats.

Comments on our model of bone stress

Our treatment of bones as fixed cantilever beams oversimplifies the complexity of *in vivo* quadruped bone stresses (Blob and Biewener, 2001), but is useful for contrasts of bending stress among species. These comparisons are appropriate if the magnitude of hindlimb force is a constant proportion of body weight across species, as has been shown for terrestrial mammals (Biewener, 1991), and if forces are exerted at a consistent angle (θ) to the long axis of the bone across species. The latter assumption can only be tested through measurements from a broad range of species. Our measurements of θ in the poorly crawling *P. parnellii* indicate that this species does not employ the advantage that could be gained by aligning large forces with the long axis of the tibia. Bats would be able to drastically reduce the stresses on their hindlimb bones by adjusting the positions of their limbs during locomotion. As a result, differences in kinematic strategies among species could influence the relative magnitudes of hindlimb stresses.

The difference in cortical thickness between the hindlimb bones of *D. rotundus* and *M. lucifugus* demonstrates that internal structure varies among bat species, and may therefore be an important component of hindlimb strength. Because we do not have data on more species, we do not know whether cortical thickness differs in vampire bats compared with all other species, or whether the cortical thickness of vampire leg bones is within the range of values demonstrated by other bats. However, since we assumed

that relative cortical thickness is constant when it appears to be less in *D. rotundus*, our model likely overestimates the strength of vampire bat limb bones. A more thorough survey of cortical thickness among bats would permit an improved model, where leg bones could be modeled as hollow beams of known thickness. In vivo stresses on the bones of bats are complex during flight (Swartz et al., 1992), and are likely also complex when bats crawl. To understand how stresses in bones compare among species, a detailed analysis should be made of bone structure from micro CT-scans, and then combined with kinematic and muscle activation data from each species. This would permit analyses to include stresses that result from internally produced forces, which are not considered in this study.

Form and function in the non-aerial locomotion of bats

Why are some bats better at walking than others?

As suggested by Strickler (1978), the proportions of the shoulder muscles may be important determinants of walking ability. Also, the fine motor control associated with the slow movements of walking may require specific muscle fibre types that are absent from most bats. The pectoralis muscles of *D. rotundus* and *D. youngi* contain four fibre types, including three fast-twitch types (IIa, IIb, IIc) and one slow-twitch type (I) (Hermanson et al., 1993; Hermanson et al., 1998). The pectoralis of all other bats studied to date possess between one and three fibre types, and none possess type I fibres (Brigham et al., 1990; Hermanson et al., 1993). Such an array of fibre types in terrestrially adept species may provide the functional capacity to coordinate support and movement while meeting the power requirements of flight (Hermanson et al., 1993). Although the pectoralis muscles of bats like

P. parnellii can supply the power necessary for flight, they might be incapable of the slow, coordinated contractions necessary to hold the body steady above the ground.

The hypothesis that type I fibres facilitate non-aerial locomotion by bats is supported by the fact that the type I fibres of *D. rotundus* are present in the m. pectoralis abdominis (Hermanson et al., 1993). Strickler (1978) listed this muscle as a major humeral retractor, important to non-aerial locomotion. The presence or absence of type I fibres from bats that crawl well but which are not closely related to the vampire bats will help to resolve the importance of that character to walking. It should be noted that many shrews (Insectivora) walk and run without any type I fibres at all (Hermanson et al., 1996; Savolainen and Vornanen, 1995; Suzuki, 1990), but that the type II fibres of insectivorans may differ in their contractile speed and rate of fatigue from those of bats (Goslow, 1985).

The terrestrial abilities of the vampire bats are impressive. *D. rotundus* are known to walk or hop forward, sideways, backward (Altenbach, 1979), and perform unique flight-initiating jumps during which vertical forces equal to 9.5 times body weight are exerted by the forelimbs in under 30 ms (Schutt et al., 1997). Comparable kinematic observations are lacking for other walking species, including the highly terrestrial New Zealand Short-tailed Bats (*M. tuberculata*), which diverged from *Desmodus* ca. 47 mya, and almost certainly evolved their terrestrial habits independently of the vampires (Teeling et al., 2003). Comparative studies have not been performed to determine whether these convergent taxa perform coordinated locomotion in the same ways. The lack of such data makes it difficult to isolate the mechanisms that enable walking in some bats, or prevent it in others, but

our experimental results demonstrate that the apparent strength of the hindlimb bones does not determine walking ability.

Ecological and behavioural correlates of walking ability

Our data (Table 1) reveal that among the bats included in this study the tibiae of *P. vampyrus* and *H. commersoni* are likely to be the most susceptible to breaking from non-aerial locomotion. If either of these species is able to walk, we predict that they do so by carefully restricting the orientation of force applied to the tibia, or by avoiding higher-level load application to the hindlimbs, perhaps by dragging them passively behind. *H. commersoni* roost in caves and trees, and take large flying insects by hawking (Vaughan, 1977), while *P. vampyrus* roost and forage in trees (Goodwin, 1979). *P. vampyrus* have been observed in captivity to crawl quickly to a vertical surface when placed on a concrete floor (M. O'Brien, personal communication), and similar observations have been made of this species in the wild (J. Epstein, personal communication). Since the tibiae of *P. vampyrus* are less robust than all other bones included in this study, and since non-aerial locomotion has been observed in this species, we can be certain that a slender hindlimb skeleton does not, in itself, prevent crawling by bats. Those bats that do not crawl at all must be limited by some other factor.

The inability to crawl occurs in several bat species. For example, it has been reported that adult *Leptonycteris* sp. and *Macrotus* sp. (Phyllostomidae) are incapable of crawling, although juveniles of both species do crawl (Dietz, 1973). The fact that *N. tumidirostris* did not attempt to crawl in our enclosure suggests that adults of this species may also be

incapable of terrestrial locomotion. *N. tumidirostris* frequently alighted from the floor of our cage in a single jump, so terrestrial locomotion may not be necessary for this species. Vaughan (1959) made similar observations of *Macrotus californicus*, which would not attempt to crawl, but instead launched into flight directly from the ground. The ability to initiate flight from a horizontal surface is probably a prerequisite for loss of crawling ability, although this ability in itself does not restrict crawling, as is demonstrated by *D. rotundus*.

We did not observe successful flight-initiating jumps by *P. parnellii*. Vaughan (1959) similarly observed that free-tailed bats (Molossidae) could only initiate flight once they had climbed to a suitable height. *P. parnellii* roost in large colonies within caves and mines, where individuals can number in the thousands (Herd, 1983). Each night they fly close to the ground through cluttered environments at speeds averaging $4.9 \text{ m}\cdot\text{s}^{-1}$ to regions where they feed aerially on insects (Bateman and Vaughan, 1974; Kennedy et al., 1977). When bats accidentally strike an obstacle, such as another bat in the cave or a branch in their foraging territory, they are likely to fall to the ground. Since *P. parnellii* do not take flight from the ground, the ability to shuffle, however awkwardly, provides a distinct advantage for bats of this species.

There is a broad diversity in crawling ability represented by Chiroptera. The terrestrial abilities of *P. parnellii* represent a mid-way point between the complete absence of crawling by *N. tumidirostris* and the agility of *D. rotundus* and *D. youngi*. Whatever the advantages of long, thin legs to bats, it appears from our data that in the majority of species reduction of the hindlimb robustness has not exceeded the mechanical requirements of non-

Table 1: Mean hindlimb bone dimensions and predicted stresses of museum specimens in 50 species. Stress estimates are calculated using Equation 3, as explained in the text. Symbols used are as follows: M_b =body mass, λ_f =femur length, δ_f =femur diameter, σ_f =relative femur stress, λ_t =tibia length, δ_t =tibia diameter, σ_t =relative tibia stress. Relative stresses are dimensionless values based on the estimated stress of *D. youngi* tibiae, which we arbitrarily assigned a value of 1.0. Those specimens with broken or missing tibiae are denoted by an asterisk. Sources used for body-mass estimates are denoted by superscripts (a: Norberg, 1981; b: Hudson and Wilson, 1986; c: Best et al., 1996; d: Kiser, 1995; e: Jones and MacLarnon, 2004; f: Burnett et al., 2001; g: Hosken et al., 2001; h: Storz and Kunz, 1999; i: Hermanson and O'Shea, 1981; j: Jones, 1977; k: Wilkins, 1987).

	N	M_b (kg)	λ_f (mm)	δ_f (mm)	σ_f	λ_t (mm)	δ_t (mm)	σ_t
Emballonuridae								
<i>Rhynchonycteris naso</i>	2	0.0039 ^a	14.0	0.6	0.50	13.9	0.4	1.81
<i>Saccopteryx bilineata</i>	2	0.0075 ^a	16.5	0.7	0.57	19.6	0.7	0.84
Megadermatidae								
<i>Lavia frons</i>	1	0.0320 ^a	24.2	1.1	1.18	29.0	0.9	2.65
<i>Macroderma gigas</i>	1	0.1500 ^b	45.3	1.9	1.89	45.6	1.5	3.94
Molossidae								
<i>Eumops perotis</i>	2	0.0590 ^c	25.8	1.5	0.83	19.3	1.0	2.00
<i>E. underwoodi</i>	1	0.0540 ^d	24.1	1.7	0.51	20.9	1.5	0.65
<i>Molossus ater</i>	1	0.0317 ^e	18.1	1.4	0.37	15.6	1.0	1.01
<i>M. bondae</i>	2	0.0179 ^f	11.9	0.9	0.51	10.6	*	*
<i>M. molossus</i>	4	0.0161 ^a	14.5	1.0	0.43	12.8	2.0	0.04
<i>Mops condylurus</i>	2	0.0330 ^g	14.8	1.1	0.60	13.1	2.0	0.10
<i>Tadarida brasiliensis</i>	3	0.0122 ^a	14.3	0.9	0.44	11.6	0.7	0.78
Mormoopidae								
<i>Mormoops megalophylla</i>	2	0.0155 ^e	25.5	1.0	0.64	21.8	0.6	2.75
<i>Pteronotus parnellii</i>	5	0.0191 ^e	20.9	1.0	0.79	18.9	0.7	2.06
Natalidae								
<i>Natalus stramineus</i>	1	0.0054 ^g	20.0	0.8	0.45	18.6	0.5	1.40
Noctilionidae								
<i>Noctilio albiventris</i>	11	0.0356 ^e	20.2	1.5	0.41	19.9	1.3	0.52
<i>N. leporinus</i>	2	0.0590 ^a	32.1	2.0	0.42	37.7	1.5	1.22
Nycteridae								
<i>Nycteris macrotis</i>	1	0.0115 ^a	24.1	0.8	0.95	21.8	0.8	1.04

Table 1 (continued)

	<i>N</i>	<i>M_b</i> (kg)	λ_f (mm)	δ_f (mm)	σ_f	λ_t (mm)	δ_t (mm)	σ_t
Phyllostomidae								
<i>Artibeus jamaicensis</i>	5	0.0470 ^a	22.6	1.3	0.83	19.3	1.0	1.59
<i>A. phaeotis</i>	2	0.0104 ^a	14.9	0.8	0.58	13.1	0.6	0.98
<i>A. toltecus</i>	1	0.0165 ^e	15.6	0.8	0.88	12.7	1.2	0.21
<i>Desmodus rotundus</i>	7	0.0285 ^a	22.5	1.9	0.16	23.4	1.5	0.35
<i>Diaemus youngi</i>	1	0.0347 ^e	21.1	1.4	0.52	21.9	1.1	1.00
<i>Glossophaga soricina</i>	1	0.0146 ^a	13.9	0.7	1.04	12.8	0.6	1.51
<i>Macrotus californicus</i>	1	0.0141 ^a	21.4	0.9	0.86	20.2	0.7	1.46
<i>Phyllostomus hastatus</i>	6	0.1100 ^a	31.8	2.1	0.71	28.7	1.6	1.31
<i>Uroderma magnirostrum</i>	1	0.0294 ^e	16.0	0.8	1.95	14.8	1.1	0.66
<i>Vampyrops helleri</i>	1	0.0133 ^a	13.2	0.7	0.90	12.6	0.7	0.86
Pteropodidae								
<i>Cynopterus brachyotis</i>	1	0.0321 ^e	19.0	1.2	0.70	23.4	1.1	1.14
<i>C. sphinx</i>	1	0.0465 ^h	18.8	1.2	0.91	24.3	0.9	2.64
<i>Eidolon helvum</i>	2	0.2740 ^a	37.2	3.0	0.69	44.9	2.0	2.90
<i>Eonycteris spelaea</i>	3	0.0549 ^e	19.4	1.5	0.56	25.6	1.1	1.77
<i>Epomorphorus</i>								
<i>wahlbergi</i>	1	0.0870 ^a	17.4	1.6	0.65	26.6	1.1	3.05
<i>Pteropus alecto</i>	1	0.5950 ^e	61.5	3.6	1.38	*	*	*
<i>P. giganteus</i>	1	0.3470 ^a	54.2	3.7	0.68	57.9	2.5	2.26
<i>P. hypomelanus</i>	2	0.3900 ^e	51.4	3.5	0.80	55.9	2.8	1.76
<i>P. vampyrus</i>	1	1.0781 ^e	71.2	4.0	2.17	82.4	2.7	7.93
Rhinolophidae								
<i>Hipposideros</i>								
<i>commersoni</i>	1	0.1300 ^a	32.1	1.6	1.97	34.3	1.0	7.83
Rhinopomatidae								
<i>Rhinopoma</i>								
<i>microphyllum</i>	1	0.0271 ^e	22.3	1.0	1.06	23.4	0.8	2.64
Vespertilionidae								
<i>Antrozous pallidus</i>	11	0.0189 ⁱ	19.6	1.1	0.45	19.9	1.0	0.69
<i>Corynorhinus rafinesquii</i>	3	0.0101 ^j	17.9	0.8	0.66	19.1	0.6	1.33
<i>Eptesicus fuscus</i>	2	0.0166 ^a	15.2	0.8	0.95	15.9	0.7	1.35
<i>Lasionycteris</i>								
<i>noctivagans</i>	1	0.0106 ^a	14.9	0.8	0.66	15.4	0.6	1.33
<i>Lasiurus borealis</i>	1	0.0131 ^a	17.1	0.9	0.54	17.7	0.7	1.19
<i>L. seminolus</i>	2	0.0122 ^k	17.8	0.9	0.55	17.6	*	*
<i>Myotis grisescens</i>	2	0.0102 ^a	14.3	0.8	0.55	16.7	0.7	1.09
<i>M. keeni</i>	1	0.0070 ^a	13.4	0.7	0.60	14.8	0.6	0.84
<i>M. lucifugus</i>	2	0.0081 ^a	13.6	0.7	0.51	15.3	0.7	0.79
<i>M. nigricans</i>	1	0.0042 ^a	11.2	0.6	0.49	12.1	0.5	0.71
<i>Nyctalus noctula</i>	1	0.0265 ^a	18.6	1.3	0.39	18.2	1.2	0.49
<i>Pipistrellus subflavus</i>	2	0.0059 ^a	12.7	0.7	0.43	13.8	0.5	0.99

aerial locomotion. Perhaps the requirements of crawling have constrained their reduction in those species that cannot initiate flight from the ground.

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CHAPTER 3: INDEPENDENT EVOLUTION OF RUNNING IN VAMPIRE BATS*

Introduction, Results, and Discussion

Most tetrapods have retained terrestrial locomotion since it evolved in the Palaeozoic era (Gambaryan, 2002; Parchman et al., 2003), but bats have become so specialized for flight that they have almost lost the ability to manoeuvre on land at all (Riskin et al., 2005; Vaughan, 1959). Vampire bats, which sneak up on their prey along the ground, are an important exception. Here we show that common vampire bats can also run by using a unique bounding gait, in which the forelimbs instead of the hindlimbs are recruited for force production as the wings are much more powerful than the legs. This ability to run seems to have evolved independently within the bat lineage.

Bats (Chiroptera) are the only mammals that fly, so their bodies differ from those of terrestrial mammals. As a result, most grounded bats can only shuffle awkwardly from a sprawled position (Riskin et al., 2005). However, the common vampire bat (*Desmodus rotundus*) walks forwards, sideways

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and backwards (Altenbach, 1979), and initiates flight with a single vertical jump from standing (Schutt et al., 1997). Captive *D. rotundus* have also been found to 'hop' at speeds exceeding 2.0 metres per second (Altenbach, 1979).

To determine whether this hopping behaviour constitutes a stereotyped running gait by *D. rotundus*, we tested five adult males on a treadmill inside a Plexiglas cage. The animals used a walking gait at low treadmill speeds (0.12 to 0.56 m·s⁻¹) and a stereotyped running gait at high speeds (0.28 to 1.14 m·s⁻¹). The walking gait was similar to the typical lateral-sequence walking gait of other tetrapods (Hildebrand, 1985); however, the run was different from any gait previously described (Fig. 9). We classify this novel gait as a run because it includes a notable aerial phase.

A tetrapod typically increases its speed while walking by increasing its stride frequency. At some transition speed, animals switch to a running gait that permits a further increase in speed, but at stride frequencies that are lower than would be predicted for high-speed walking (Heglund and Taylor, 1988; Taylor et al., 1982). Our kinematic data from *D. rotundus* fit this general stride frequency-velocity relationship. In Fig. 10, the slopes of the stride-frequency– velocity regressions, which are best fits to the walking and running data, respectively, and are shown truncated at the intersection, are significantly different (*t*-test, *P* 0.0001, *n*=61). These regression lines indicate that common vampire bats, like other running tetrapods, keep their

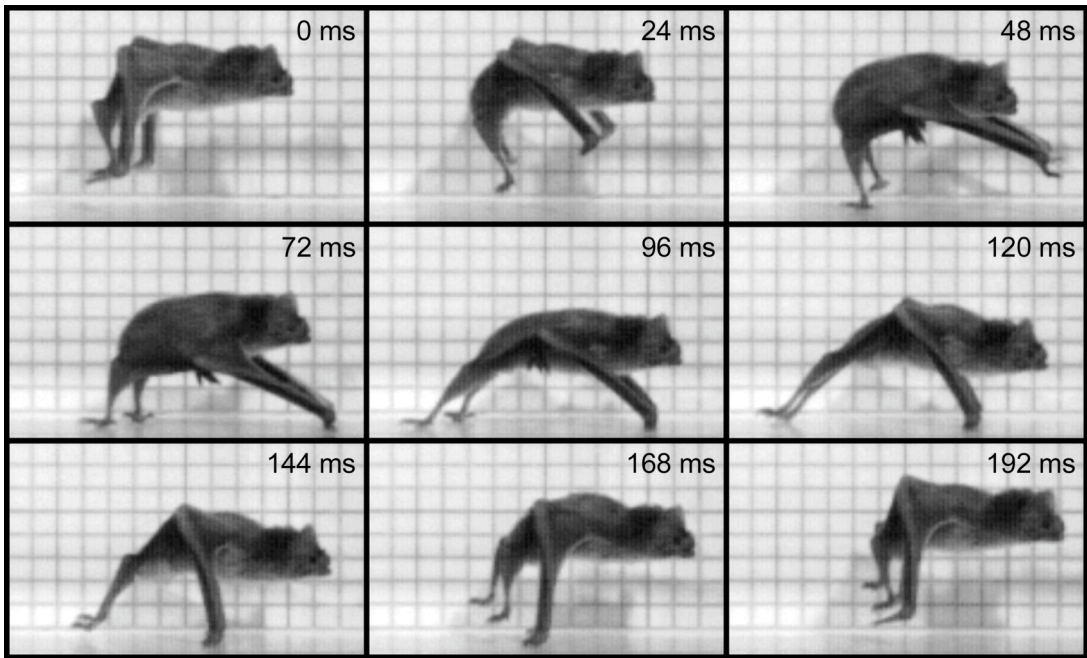


Figure 9: A vampire bat, *Desmodus rotundus*, using a running gait at $0.61 \text{ m}\cdot\text{s}^{-1}$ with a stride frequency of 4.71 Hz. Images are shown at 24-ms intervals; the background is a 1.0-cm^2 grid.

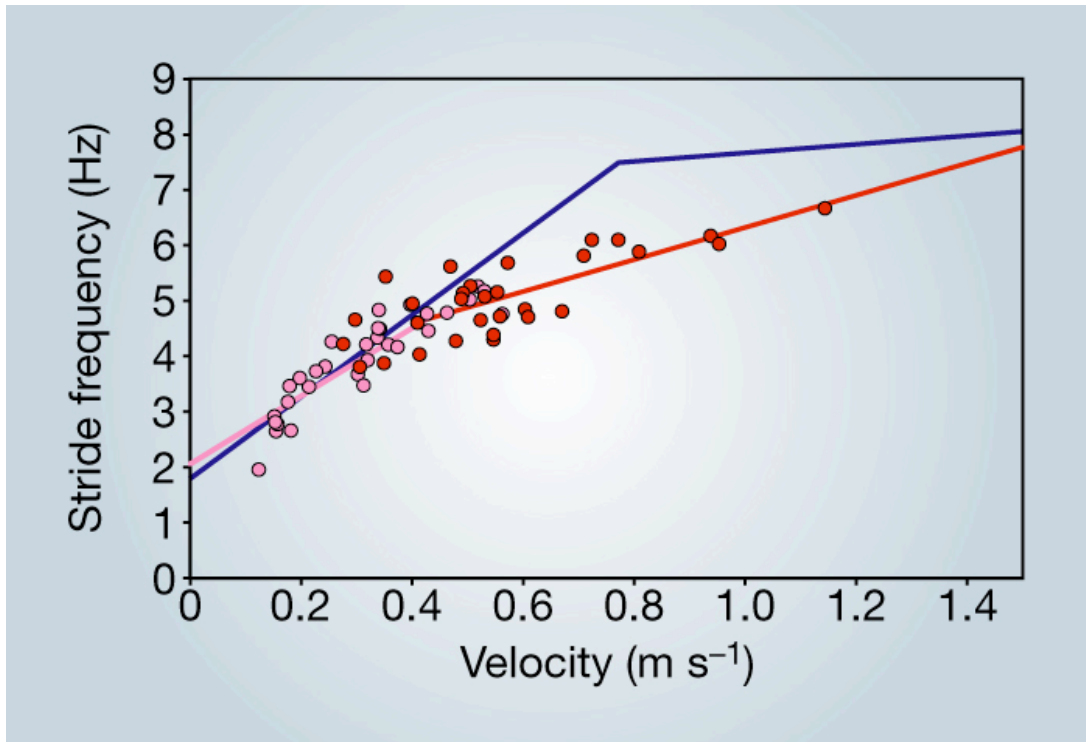


Figure 10: Stride frequency plotted against velocity for vampire bats (*Desmodus rotundus*; $n = 5$) moving on a treadmill. Pink circles, walking; red circles, running. Pink and red lines, best fits for walks and runs, respectively, truncated at their point of intersection; blue lines, best fits for walks and runs, respectively, of similarly sized (29 g) mice (data from Heglund and Taylor, 1988), shown here for comparison.

stride frequencies low by walking at low speeds and running at high speeds (Fig. 10).

The walking vampire bats used stride frequencies that were comparable to those of similarly sized terrestrial mammals (mice) over the same range of speeds (Fig. 10; blue line). When running, however, the bats used lower stride frequencies than mice (Heglund and Taylor, 1988): this could be explained by the vampire bats' long forearms, which allow longer and fewer strides to be taken during running than can be achieved by mice.

The absence of a running gait in all other bat species so far surveyed indicates that running may have been lost early in the evolution of bats, evolving afresh in the vampires at a later time. We have shown that the hopping behaviour reported for *D. rotundus* in captivity (Altenbach, 1979) is a running gait. But despite detailed knowledge of their roosting and foraging behaviour (Greenhall and Schmidt, 1988; Turner, 1975), the selective benefit of running for these bats in the wild is not known. Presumably, vampire bats are most likely to run when manoeuvring around prey animals while feeding, and they may have used the gait more before the introduction of domestic livestock to the Americas in the sixteenth century (Greenhall and Schmidt, 1988).

Methods

Capture Methods

We performed experiments on naive *Desmodus rotundus*, captured using mist nets from two localities in southwest Trinidad in July 2004. All

investigations were conducted within 24 h of capture, and in accordance with Cornell University IACUC approval and permits issued by the Ministry of Agriculture, Trinidad and Tobago.

Kinematic Recordings

For each sequence of trials, we introduced a bat to a Plexiglas cage (0.48 m length, 0.15 m width, 0.11 m height) with a variable-speed treadmill as its floor. Using a digital video camera (250 Hz) and a mirror, we recorded the bat's movements in lateral and dorsal views. In a trial, the treadmill was accelerated smoothly to a constant speed. After the subject matched its speed to that of the treadmill over several strides, the treadmill was stopped, and the bat permitted to rest for ca. 60 s. The trials were conducted over increasing speeds, until the bat showed visible signs of fatigue. Each bat was used in only one sequence of trials. Each trial was treated as a separate event for statistical analyses.

CHAPTER 4: TERRESTRIAL LOCOMOTION OF THE NEW ZEALAND
SHORT-TAILED BAT, *MYSTACINA TUBERCULATA*, AND THE COMMON
VAMPIRE BAT, *DESMODUS ROTUNDUS**

Summary

Bats (Chiroptera) are generally awkward crawlers, but the Common Vampire Bat (*Desmodus rotundus*) and the New Zealand Short-tailed Bat (*Mystacina tuberculata*) have independently evolved the ability to manoeuvre well on the ground. In this study we describe the kinematics of locomotion in both species, and the kinetics of locomotion in *M. tuberculata*. We sought to determine whether these bats move terrestrially the way other quadrupeds do, or whether they possess altogether different patterns of movement on the ground than are observed in quadrupeds that do not fly. Using high-speed video analyses of bats moving on a treadmill, we observed that both species possess symmetrical lateral-sequence gaits

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similar to the kinematically defined walks of a broad range of tetrapods. At high speeds, *D. rotundus* use an asymmetrical bounding gait that appears to converge on the bounding gaits of small terrestrial mammals, but with the roles of the forelimbs and hindlimbs reversed. This gait was not performed by *M. tuberculata*.

Many animals that possess a single kinematic gait shift with increasing speed from a kinetic walk (where kinetic and potential energy of the centre of mass oscillate out of phase from each other) to a kinetic run (where they oscillate in phase). To determine whether the single kinematic gait of *M. tuberculata* meets the kinetic definition of a walk, a run, or a gait that functions as a walk at low speed and run at high speed, we used force plates and high-speed video to characterize the energetics of the centre of mass in that species. Although oscillations in kinetic and potential energy were of similar magnitudes, *M. tuberculata* did not use pendulum-like exchanges of energy between them to the extent that many other quadrupedal animals do, and did not transition from a kinetic walk to kinetic run with increasing speed. The gait of *M. tuberculata* is kinematically a walk, but kinetically run-like at all speeds.

Introduction

Tradeoffs in locomotion

In many animals, morphology matches the mechanical requirements of locomotion to produce an effective movement system. For example, whales have body shapes that generally minimize drag in water over a broad range of swimming speeds, and choose fluke beat frequencies that

maximize efficiency while swimming (Rohr and Fish, 2004). Similarly, the bodies of dogs are well-suited to long-distance travel over land, and they use walking and running gaits that minimize the metabolic cost of locomotion for their body plans (Goslow et al., 1981). In both of these cases, evolution has resulted in morphology and behaviour that function efficiently in a single mode of locomotion. This is, however, not always the case. For example, consider the sea lion (Carnivora: Otariidae) that spends much of its time in the water, but must also manoeuvre on land. As the result of having a body well-suited to swimming after elusive prey (Fish et al., 2003), sea lions are less agile on the ground than typical terrestrial mammals, and thus move quite differently from them (Chechina et al., 2004).

In instances where animal morphology simultaneously meets the requirements of more than one form of movement, studies of form and function take on another dimension of complexity. Additionally, the issue of tradeoffs and compromise may be enlightening to investigations of morphological adaptation. Organisms that perform more than one type of locomotion offer insight into how animals might transition between modes of transportation over the course of their evolution, like the sarcopterygian fish that gave rise to tetrapods, the theropod dinosaurs that gave rise to flying birds, and the ungulates that gave rise to whales (Ashley-Ross, 1995; Dial, 2003; Gingerich, 2005).

These issues have been addressed previously in studies of tetrapods that move terrestrially and aquatically (Ashley-Ross and Bechtel, 2004; Biewener and Corning, 2001; Biewener and Gillis, 1999; Fish et al., 2001). In this study, we explore such compromises using bats (Chiroptera) as a model. Unlike walking birds, that use the forelimbs for flight and the

hindlimbs for walking, bats use all four limbs for both modes of locomotion. Bats are extremely agile in the air but compared to other mammals most bats move awkwardly on the ground (Schutt and Simmons, 2006; Vaughan, 1959, 1970), suggesting biomechanical tradeoffs between aerial and non-aerial locomotion.

Origins of terrestrial agility in two bat species

There are more than 1,100 currently recognized species of bat (Simmons, 2005), and the majority of these spend very little time traveling on the ground. Typically, when a bat accidentally falls to the ground, having struck an obstacle in flight or fallen from an overhanging roost, it either immediately launches itself directly back into flight by pressing its wings on the substrate, or shuffles to a vertical feature of the environment, climbs it, then drops into flight (Vaughan, 1959). Those bat species that forage for terrestrial prey typically do so by landing directly on their prey, rather than by chasing them down on foot (Johnston and Fenton, 2001; Ratcliffe and Dawson, 2003). A few bats move fairly well on the ground, most notably molossids and vespertilionids, but they generally fall short of the rapid bounding and hopping locomotion performed by terrestrial mammals of similar size (Biewener et al., 1981; Biewener and Blickhan, 1988; Hatt, 1932). However, the Common Vampire Bat (Phyllostomidae: *Desmodus rotundus*) and the New Zealand Short-tailed Bat (Mystacinidae: *Mystacina tuberculata*) are extremely agile crawlers, even though they are also fully capable of flight (Schutt and Simmons, 2006).

Desmodus rotundus are obligate blood-feeders, found in Mexico, Central and South America, and two Caribbean islands, where they primarily

parasitize domestic livestock, such as cattle (Turner, 1975). Terrestrial locomotion permits them to approach their hosts stealthily, and to escape if the prey animal or some other danger threatens them while feeding (Altenbach, 1979). *D. rotundus* also initiate flight with rapid and powerful jumps that enable them to attain a vertical velocity of $2.4 \text{ m}\cdot\text{s}^{-1}$ in less than 30 ms (Schutt et al., 1997). This type of rapid escape is necessary in habitats where terrestrial predators of bats are plentiful, and is especially needed by a bat that sometimes feeds with its tongue against the foot of an animal that outweighs it 14,000-fold (Greenhall, 1988).

Mystacina tuberculata are restricted to New Zealand, where they also frequently utilize terrestrial locomotion, but their ecology and behaviour are quite different from those of vampire bats. New Zealand is well-known for its flightless birds (most famously kiwis, *Apteryx* spp.) that evolved terrestrial habits in the absence of snakes or predatory mammals, prior to the arrival of invasive species with humans. Similarly, *M. tuberculata* expanded their niche from the aerial hawking and/or gleaning that typifies most bats, to include significant terrestrial foraging. *M. tuberculata* spend some 30% of their foraging time crawling, even burrowing, while searching for arthropods, fruit, nectar, and pollen (Daniel, 1976, 1979).

Common Vampire Bats are more closely related to poorly crawling bats (e.g. phyllostomids, mormoopids) than they are to New Zealand Short-tailed Bats (Teeling et al., 2003, 2005), suggesting that these taxa evolved their terrestrial behaviours independently. Both move quadrupedally, as do the majority of mammals, but the bats do so using limbs that are specialized for aerial locomotion. We were therefore interested to know whether their movement patterns are similar to those of other quadrupeds, or whether

they involve altogether different patterns. Because *D. rotundus* and *M. tuberculata* manoeuvre terrestrially so well compared with other bats, their anatomy has been the subject of several investigations (Altenbach, 1979; Dwyer, 1960a, 1962; Howell and Pylka, 1977; Riskin et al., 2005; Schutt, 1998; Schutt and Altenbach, 1997; Strickler, 1978). However, while previous studies provided descriptions and photographs of locomotion in *D. rotundus* (Altenbach, 1979; Riskin and Hermanson, 2005), they did not include many of the kinematic parameters useful for comparing their gaits with those of other tetrapods. We report several such parameters here. Also, this is the first study to report the kinematics of locomotion in *M. tuberculata*.

Describing locomotion

There are several different ways to classify gaits so that they can be compared among species, and most of these movement taxonomies include a distinction between walking and running (Ahn et al., 2004; Cavagna et al., 1976; Hildebrand, 1985; Ruina et al., 2005). As a result, there are several criteria by which to distinguish the two. In this study, we make use of kinematic and kinetic distinctions between walks and runs.

Kinematic definitions of gait

To make our observations of both bat species comparable with those of as many organisms as possible, we follow kinematic definitions of gait that have been applied to >150 genera of quadrupeds (e.g. Hildebrand, 1985). By one kinematic definition, a run is characterized by the presence of an aerial phase, where all four limbs are off the ground at some point during the stride cycle, while in a walk at least one limb touches the ground at all times. By another definition, a gait in which a limb spends more than 50% of

the stride cycle in contact with the ground (duty factor >0.5) is considered a walk, while one in which the duty factor is less than 0.5 is defined as a run (Ahn et al., 2004; Hildebrand, 1985; Hutchinson et al., 2003; Rubenson et al., 2004).

Since the footfall patterns of quadrupedal animals are largely governed by stability (Alexander, 1977; Cartmill et al., 2002), which is a biomechanical constraint that operates independently of evolutionary origins, we expected the footfall patterns of bats to fall within the range that has been described for quadrupedal animals that do not fly. Also, if bats walk the way other tetrapods do, we would expect that bats using a single kinematic gait over increasing speeds will increase their stride frequencies and decrease their duty factors (Ahn et al., 2004; Dutto et al., 2004; Fish et al., 2001; Heglund and Taylor, 1988).

Kinetic definitions of gait

In many recent studies, force plates have been used to apply kinetic (or energetic) distinctions between walking and running to a broad range of animals, including mammals, birds, reptiles, amphibians, and arthropods (Ahn et al., 2004; Blickhan and Full, 1987; Cavagna et al., 1976; Farley and Ko, 1997; Goslow et al., 1981; Griffin and Kram, 2000; Minetti et al., 1999). Specifically, a gait where kinetic energy (E_K) and gravitational potential energy (E_P) of the centre of mass (COM) oscillate out of phase is considered a kinetic walk, while one in which E_K and E_P oscillate in phase is considered a kinetic run (Cavagna et al., 1977). These kinetic definitions are motivated by ideas about the mechanisms of energy conservation employed by moving animals. In a gait where E_K and E_P oscillate out of phase, energy can be

cycled between them in a pendulum-like manner (Cavagna et al., 1977; Ruina et al., 2005). In a kinetic run, exchanges of energy between E_K and E_P (here defined as gravitational potential energy) are decreased, so more energy must either be supplied by muscles or be stored in spring-like tendons and muscles, making the energetics of running analogous to that of a bouncing ball or pogo-stick (Cavagna et al., 1977).

Confusingly, a gait that might meet the criteria of a kinematic walk might be classified as a run by kinetic nomenclature. For example, Gatesy and Biewener (1991) and Rubenson et al. (2004) have observed that the single kinematic gait of a bipedal bird can transition from a kinetic walk at low speeds to a kinetic run at higher speeds. Similar trends have also recently been noted for quadrupedal frogs by Ahn et al. (2004). Because *M. tuberculata* in this study exhibited only one kinematically distinguishable gait (see results), we sought to determine whether a range of kinetic gaits exists within that single kinematic gait. We expected that *M. tuberculata* would transition from a kinetic walk to a kinetic run with increasing speed.

Materials and methods

Capture and handling of bats

In July 2004, we captured *D. rotundus* Weid 1826 (five males; body mass $23.1 \pm \text{s.d. } 2.0$ g) from ranches in Southwestern Trinidad. In November 2004 we caught *M. tuberculata* Gray 1843 (three males, three females; 13.9 ± 0.9 g) in Fiordland, New Zealand. Each bat was used in only one sequence of force plate trials, and one subsequent sequence of treadmill trials. All experiments were performed within 24 h of capture. Protocols for

capture and experimentation were approved by the Cornell University Institutional Animal Care and Use Committee, the University of Auckland Animal Ethics Committee, the Ministry of Agriculture (Forestry Division) of Trinidad and Tobago, and the Department of Conservation of New Zealand.

Gait kinematics: treadmill trials

Treadmill design

To observe the terrestrial gaits of animals over a broad range of speeds, we placed each bat inside a custom-built Plexiglas enclosure 0.48 m long, 0.15 m wide, and 0.11 m high, with a floor consisting of a variable-speed treadmill. In a trial, the treadmill was accelerated smoothly to a constant speed. Once the bat had matched its crawling velocity to that of the treadmill, we recorded images at 250 Hz using a MotionMeter 250 digital high-speed camera (Redlake Systems, San Diego CA, USA). The camera was positioned ca. 2 m from the enclosure, and a mirror above the cage, angled 45° from horizontal, permitted us to record simultaneous lateral and dorsal views of the bat in each camera frame. Up to 8 s of video were recorded, then the treadmill was stopped and the bat permitted to rest for ca. 60 s before the next trial. We conducted trials over increasing speeds until either the subject appeared fatigued, or we were unable to further increase its speed.

Analyses

To measure speed and stride frequency, we recorded the time taken to complete the largest possible integer number of stride cycles in a trial. Stride frequency was calculated as the number of stride cycles divided by

this period. We measured speed by adding the change in position of the bat's nose to the change in position of markers on the treadmill surface, both relative to a stationary object, and dividing their sum by the same period.

To see how gaits changed kinematically with speed, we selected a single stride cycle sequence from each trial, beginning and ending with left hind footfall. From it, we observed the timing of footfall and foot lift events, and recorded whether or not an aerial phase occurred. Duty factors of the two forelimbs were averaged in the cycle, as were those of the hindlimbs. The two kinematic gaits of *D. rotundus* (walking and bounding) were easily distinguished by sight, and analysed separately. *M. tuberculata* used only one kinematically distinguishable gait (walking), so all trials for that species were analysed together.

It is possible that *M. tuberculata* do bound at high speeds, and did not do so in our study because the treadmill moved too slowly. To ensure that we observed locomotion by *M. tuberculata* at sufficiently high velocities, we compared the greatest speeds of *M. tuberculata* on the treadmill to the range of speeds at which *D. rotundus* used the walking and bounding gaits. To correct for the nearly two-fold difference in body mass between the two species, we compared them using a dimensionless descriptor of movement called Froude number (Fr). Animals with similar body plans transition between gaits at equivalent Froude numbers across broadly varying body sizes (Alexander and Jayes, 1983). Therefore if *M. tuberculata* walked at Froude numbers for which *D. rotundus* exclusively bounded then we would infer that the bounding gait is not used by *M. tuberculata* at any speed.

Froude number is defined as $Fr = v^2 \cdot g^{-1} \cdot l^{-1}$, where v is velocity, g is the gravitational constant ($g = 9.81 \text{ m} \cdot \text{s}^{-2}$), and l is hip height (Alexander and

Jayes, 1983). We use the mean tibia lengths of animals in our study as a proxy for l (26.8 mm in *D. rotundus*, and 16.9 mm in *M. tuberculata*), since when walking quadrupedally, bats hold the femora somewhat horizontally and the tibiae roughly vertical (Schutt and Simmons, 2006). In most tetrapods, shoulder height is roughly equivalent to hip height, but in *D. rotundus* and *M. tuberculata* the shoulder joint is much higher than the hip. We therefore only use Froude analysis to compare these bat species to one another, and do not assume dynamic similarity between the gaits of bats and those of other tetrapods.

Gait kinetics of New Zealand Short-tailed Bats: force plate trials

Force plate design, calibration, and use

Recordings of COM energetics in *M. tuberculata* were made in the same Plexiglas enclosure as that used for the treadmill trials, but the treadmill was replaced with two serially-set force platforms in the centre of the enclosure, flush with Plexiglas over the rest of the floor. The Plexiglas floor and the honeycombed fiberfoam surfaces of the force plates both appeared to provide sufficient friction for quadrupedal locomotion. We only observed the feet of bats slipping in a few instances where bats jumped, and these events were not included in our analyses.

Each force plate was 74.6 mm long, and spanned the width of the enclosure (155 mm). The plates independently measured the ground reaction forces of crawling bats in three directions, to which we refer throughout this paper as fore-aft (the axis parallel to the long-axis of the cage), mediolateral (the orthogonal horizontal axis), and vertical.

The force plates used in this study were built based on designs by Heglund (1981) and Biewener and Full (1992). A detailed description of plate design and construction are provided by Riskin et al. (2005). Each plate had resonant frequencies ≥ 128 Hz in all three directions, permitting reliable event records on the order of 7.8 ms. On each recording day the force plates were calibrated for load response in each direction, and demonstrated linear correlations of force to output voltage over a range of forces three-fold greater than the body weights of our largest animals ($r^2 > 0.999$). Electronic drift in the baseline output of the force plates was corrected in each individual trial by sampling the signal of unloaded plates (zero force) within 10 s of data collection. Crosstalk was $\leq 7\%$ between vertical and horizontal channels, and $\leq 16\%$ between horizontal channels. Force plate recordings were filtered with a 50-54 Hz Butterworth bandstop filter to remove AC noise (ca. 52 Hz in New Zealand), and with a Butterworth lowpass filter of 25 Hz to improve the signal to noise ratio overall. Signals from the two plates were summed for all calculations.

In a trial, we encouraged a bat to cross the force plates by blowing on it through a straw. As the bat crossed the plates, we recorded ground reaction forces at 1000 Hz in each of three directions, and simultaneously recorded video at 250 Hz in lateral and dorsal views. Video and force-plate signals were synchronised in the manner used by Riskin et al. (2005). The 250 Hz square wave emitted by the master/slave port of the video camera powered an LED visible in the camera frame, and was simultaneously recorded to a computer with the force recordings. The manual interruption of that signal by means of a hand-held switch during each trial permitted us to synchronise video sequences to force-plate output with a resolution of 4 ms.

Calculations of COM energetics

From each force plate trial, we isolated a single stride cycle, beginning and ending with a hind footfall, where the bat's body weight was completely supported by the force plates. From it, we calculated the energetics of the COM. Only one stride cycle was used from each trial.

Forces in fore-aft and mediolateral directions, and vertical force minus the product of mass and the gravitational constant (g), were divided by the animal's body mass to obtain instantaneous acceleration of the COM in three dimensions. Acceleration in each direction was then integrated with respect to time to calculate instantaneous velocity, and vertical velocity was integrated to determine the height of the COM throughout the trial.

To obtain constants for the integrations of acceleration (initial velocity values), we used a custom-made program in Matlab 7.0.1 (MathWorks Inc., Natick, MA, USA) to digitize the movement of the nose tip over the 10 camera frames (0.04 s) prior to the beginning of the stride cycle. A linear least squares best-fit line was calculated for both the fore-aft and mediolateral movements over time, to produce initial velocity estimates for that trial. Unfortunately, changes in the pitch of the body did not allow reliable estimates of initial vertical velocity in the same manner. Therefore, we selected an initial vertical velocity such that the calculated net change in height of the COM based on force recordings would match the observed change in the height of the nose from the beginning to the end of the trial. To ensure accuracy, calculated patterns of increase and decrease in calculated COM height over the course of the entire trial were checked against changes in the height of the bat's body in videos. The constant for integration of vertical velocity (initial height) was chosen as zero.

Kinetic energy in the fore-aft direction was calculated using the equation $E_{KF}=0.5 \cdot m v_F^2$, where m is the mass of the animal and v_F is forward velocity. Mediolateral and vertical kinetic energies (E_{KL} and E_{KV} , respectively) were calculated analogously. We defined total kinetic energy as $E_K=E_{KF}+E_{KL}+E_{KV}$, and gravitational potential energy as $E_P=mgh$, where h is the height of the COM. Total energy was defined as $E_{TOT}=E_K+E_P$.

Descriptions of COM energetics

Where E_K and E_P of the COM oscillate in serial sinusoidal patterns of similar frequency, the ‘phase shift’ between them reveals information about the degree to which energy might be exchanged in a pendulum-like manner. Although this statistic is frequently reported in studies of this kind (Ahn et al., 2004; Cavagna et al., 1977; Farley and Ko, 1997), we do not present it here because we did not observe clear sinusoidal changes of E_K or E_P from trial to trial in *M. tuberculata*.

‘Percent congruity’ (%Congruity), calculated as the percentage of time taken to complete the stride cycle for which E_K and E_P increased together or decreased together, to the exclusion of time where the product of their slopes was negative (Ahn et al., 2004), was calculated for all trials. If animals use a pendulum-like exchange of E_K and E_P , %Congruity should be near zero. If instead the kinetics are similar to those of a bouncing ball, %Congruity should approach 100%.

Percent recovery (%Recovery), has been widely used as a descriptive statistic of the potential for exchange between E_K and E_P for the stride cycle of an animal (e.g. Zani et al., 2005), so we recorded it for *M. tuberculata*. Percent Recovery was calculated as

$$\% \text{Recovery} = \frac{\Sigma \Delta E_K + \Sigma \Delta E_P - \Sigma \Delta E_{TOT}}{\Sigma \Delta E_K + \Sigma \Delta E_P} \times 100$$

where $\Sigma \Delta E$ is the sum of positive increments in a given component of energy over the course of the stride cycle (Cavagna et al., 1977). Percent Recovery for a pendulum-like kinetic walk should approach 100%, since $\Sigma \Delta E_{TOT}$ should approach zero if energy is tightly recycled between E_K and E_P . Percent Recovery for a bouncing ball-like kinetic run, should approach zero. If *M. tuberculata* use a kinetic walk at low speeds and kinetic run at high speeds, %Congruity would increase with increasing speed, while %Recovery would decrease.

Results

Treadmill Trials: Common Vampire Bats

Behaviour

All *D. rotundus* used in this study took only a matter of minutes to train on the treadmill. When the treadmill belt began moving, they quickly learned to move against its direction, and to sustain constant speed until it was stopped. In later trials, bats would make long leaps toward the front of the treadmill, stand on the moving floor until they came close to the back of the cage, then jump again. We interpreted this pattern of behaviour as the result of fatigue, and ceased trials with a given individual once it was observed. We recorded 61 treadmill trials (31 walking, 30 bounding) from five individuals over speeds ranging from 0.12 to 1.14 m·s⁻¹. The speed to stride-frequency relationship for those trials was reported elsewhere (Riskin and Hermanson, 2005). We were only able to resolve footfall patterns in 28

walking and 21 bounding trials, but the speeds and kinematic gaits from all 61 trials were used in this study for comparison to the velocities of *M. tuberculata*.

Lateral-sequence walking gait

At low speeds, *D. rotundus* used a lateral sequence gait, to a maximum speed of $0.56 \text{ m}\cdot\text{s}^{-1}$. As the left forelimb moved forward, so did the right hindlimb, and vice versa (Fig. 11a,d). Walking *D. rotundus* kept the body at a relatively constant height, so that it did not bounce, but instead moved cat-like in a straight horizontal line, as has been reported previously (Altenbach, 1979).

During the lateral sequence gait, at least one limb remained in contact with the ground at all times. Forelimb duty factors ($0.72 \pm \text{s.d. } 0.07$) were significantly greater (paired- $t=6.09$, d.f.=27, $P<0.0001$) than those of the hindlimbs (0.62 ± 0.06), and duty factors of the forelimbs and hindlimbs both exceeded 0.5 ($t=15.86$ and 10.68 , respectively, d.f.=27, $P<0.0001$). Duty factor decreased with speed in the forelimbs ($t=-2.72$, $P=0.012$; $r^2=0.22$), but only very slightly, and hindlimb duty factor decreased with speed, but not significantly ($t=-1.88$, $P=0.07$, $r^2=0.12$; Fig. 12a).

Bounding Gait

At speeds of 0.28 to $1.14 \text{ m}\cdot\text{s}^{-1}$ on the treadmill, *D. rotundus* used a bounding gait that included a dramatic aerial phase (Fig. 11b,e). This range

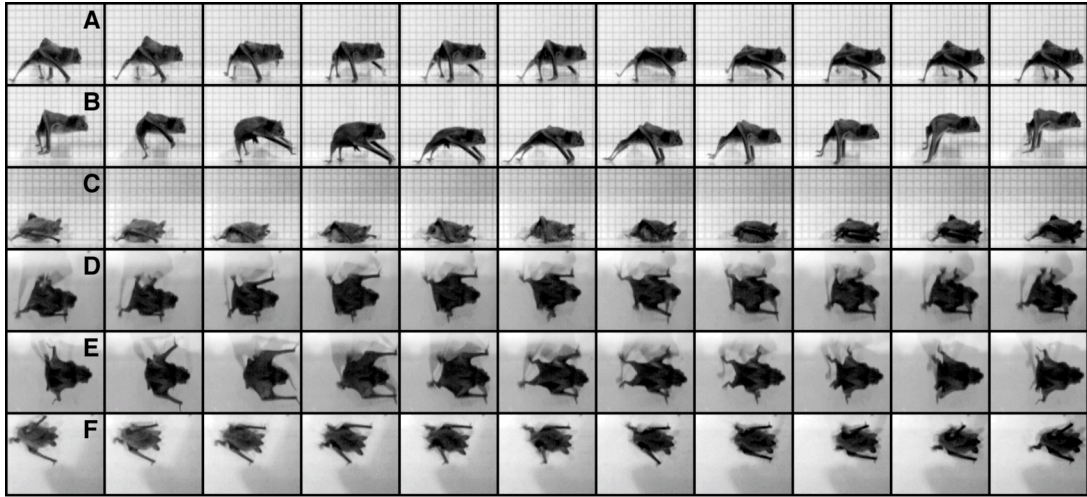


Figure 11: Representative stride cycles on the treadmill of *D. rotundus* in lateral view (a) walking at $0.12 \text{ m}\cdot\text{s}^{-1}$, (b) bounding at $0.60 \text{ m}\cdot\text{s}^{-1}$, and (c) *M. tuberculata* moving at $0.35 \text{ m}\cdot\text{s}^{-1}$. The time between frames differs among the three sequences (40, 24, and 16 ms, respectively). The background is a 1 cm^2 grid. Dorsal views of the same three sequences are shown in d, e, and f, respectively.

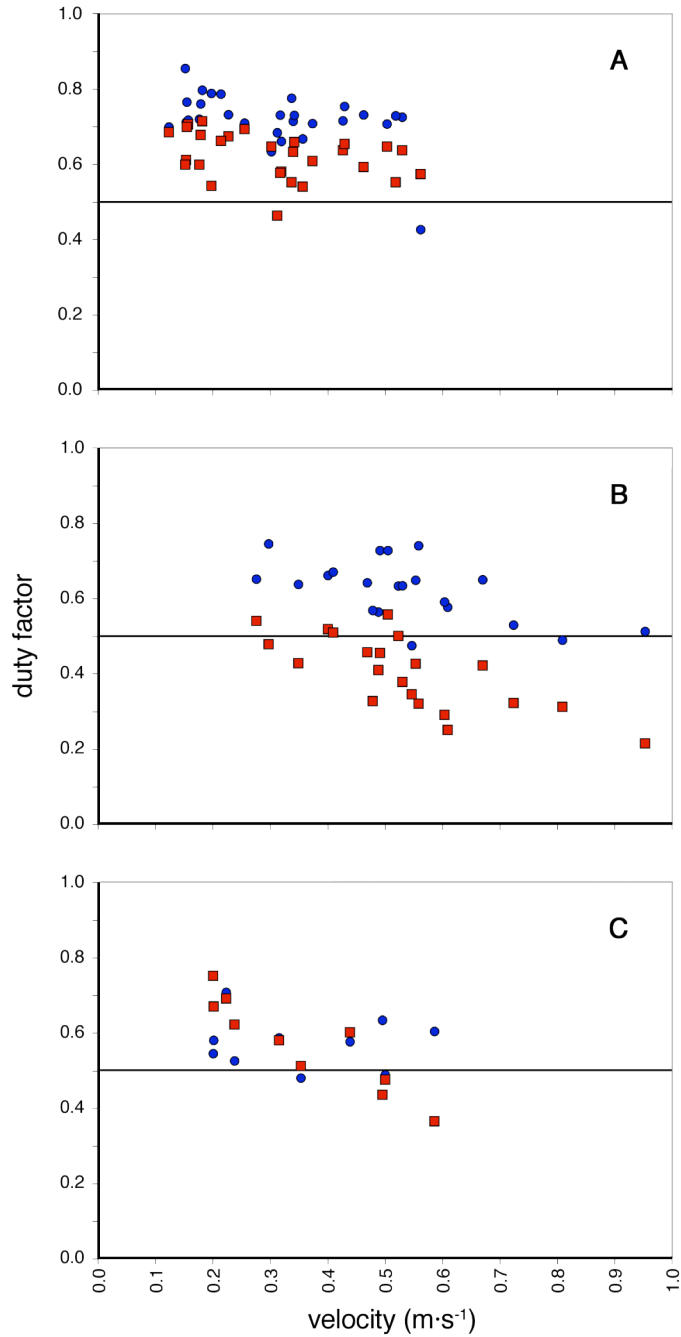


Figure 12: Duty factor (the proportion of a stride cycle for which a given limb is in contact with the ground) of treadmill trials for (a) walking *D. rotundus*, (b) bounding *D. rotundus*, and (c) *M. tuberculata*. Blue circles represent the means of left and right forelimbs in each trial, and red squares the means of hindlimbs. Each plot includes a horizontal line at duty factor = 0.5, the kinematic separation point between walks (duty factor >0.5) and runs (duty factor <0.5; Hildebrand, 1976).

of speeds overlaps with the upper 50% of speeds at which lateral-sequence walks were used in other trials, and extends into a range of speeds at which walking was not observed. During bounding, duty factors were greater than 0.5 ($t=7.00$, d.f.=20, $P<0.0001$) in the forelimbs (0.62 ± 0.08), less than 0.5 ($t=-4.56$, d.f.=20, $P<0.0001$) in the hindlimbs (0.40 ± 0.10), and decreased with increasing speed in both the forelimbs ($t=-3.27$, $P=0.004$, $r^2=0.36$) and hindlimbs ($t=-4.71$, $P=0.0002$, $r^2=0.54$; Fig. 12b).

Treadmill trials: New Zealand Short-tailed Bats

Behaviour

In general, we were unable to train *M. tuberculata* to move predictably against the motion of the treadmill within the single testing period to which each was subjected, and were unable to extend the training period due to their endangered status. When the floor began moving, bats typically sat still, forcing us to stop the treadmill before the bat reached the end of the enclosure. In those instances where the bat did travel on the moving treadmill, it seemed as likely to move with the direction of floor movement as against it. Nevertheless, we were able to glean 10 trials in which a bat moved at constant speed for at least three sequential stride sequences, from among five bats over speeds ranging from 0.20 to 0.59 m·s⁻¹. Although *M. tuberculata* sometimes made single jumps similar to the flight initiating jumps of vampire bats, we never observed any individuals jumping sequentially like bounding *D. rotundus* did.

Lateral-sequence gait

At all treadmill speeds, *M. tuberculata* (Fig. 11c,f) used a lateral-sequence walk in which stride frequency increased with increasing speed ($t=4.38$, $P=0.002$; $r^2=0.71$; Fig. 13). In general, the patterns of limb movement were consistent between trials. However the vertical movements of the body varied tremendously in frequency and amplitude from trial to trial, and did not appear to change in a predictable pattern with the movement of the limbs.

The lateral sequence walk of *M. tuberculata* did not include an aerial phase. Duty factors of forelimbs and hindlimbs were not significantly different (paired- $t=-0.05$, d.f.=9, $P=0.96$), and were generally greater than 0.5 ($t=3.30$, d.f.=9, $P=0.005$ and $t=1.79$, d.f.=9, $P=0.053$, respectively). Duty factors of the hindlimbs decreased with increasing speed ($t=-6.58$, $P=0.0002$, $r^2=0.84$) but those of the forelimbs did not change with speed ($t=-0.19$, $P=0.86$, $r^2=0.004$; Fig. 12c).

We do not believe that *M. tuberculata* perform the bounding run, since they traveled without bounding at Froude numbers (and velocities) for which *D. rotundus* used the bounding gait exclusively. The greatest speed of *M. tuberculata* on the treadmill ($Fr = 2.1$, $v=0.59 \text{ m}\cdot\text{s}^{-1}$) exceeds the top walking speed of *D. rotundus* ($Fr = 1.2$, $v=0.56 \text{ m}\cdot\text{s}^{-1}$), and lies well within the range of speeds at which *D. rotundus* used a bounding gait ($Fr = 0.3-4.9$, $v=0.28-1.14 \text{ m}\cdot\text{s}^{-1}$).

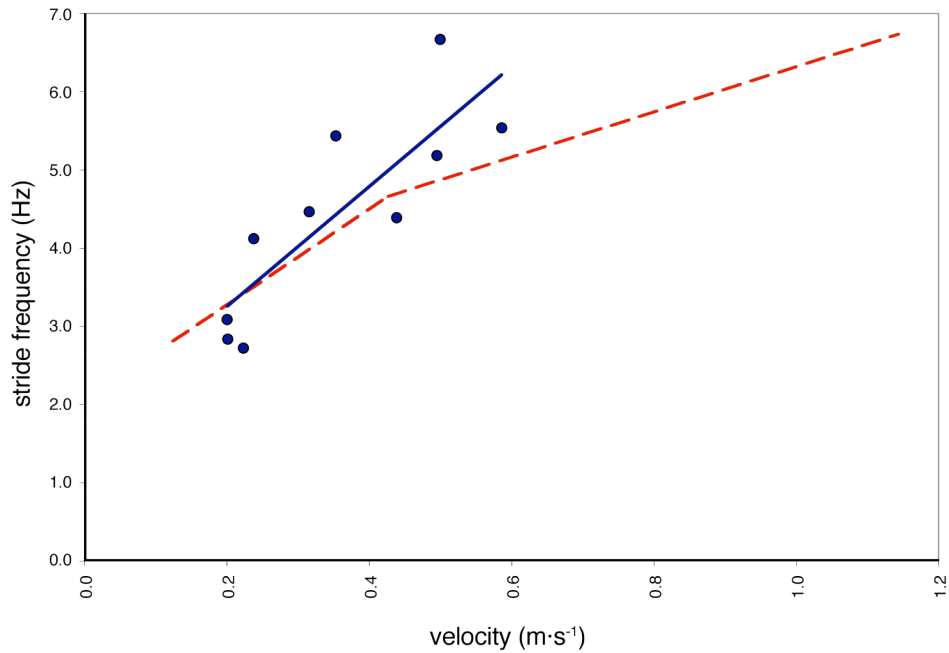


Figure 13: The gait of *M. tuberculata* (blue) demonstrates a linear increase in stride frequency with speed, just as the gaits of many other tetrapods do (Heglund and Taylor, 1988). The dashed red lines represent the linear best fit regressions for walking (left) and bounding (right) gaits of *D. rotundus*, truncated at their point of intersection (from Riskin and Hermanson, 2005).

Force plate trials: New Zealand Short-tailed Bats

We analysed 24 trials from five individuals, in which animals moved at speeds of 0.13 to 0.95 m·s⁻¹ across the force plates. Bats on the stationary force plates demonstrated similar variability in vertical body movement relative to footfall pattern from trial to trial as they did on the moving treadmill, and this was evident in plots of E_K and E_P over the course of each trial (Fig. 14).

Across trials, the magnitude of changes in E_K (1.54 ± 0.86 mJ) was not significantly different from the magnitude of changes in E_P (1.47 ± 0.91 mJ; paired- $t=0.39$, $P=0.69$). As speed increased, changes in E_{TOT} (2.35 ± 1.36 mJ) increased overall ($t=2.25$, $P=0.03$, $r^2=0.19$), but not every component of E_{TOT} did. Bats increased E_{KF} ($t=2.31$, $P=0.03$, $r^2=0.20$) and E_{KV} ($t=2.89$, $P=0.009$, $r^2=0.28$) with speed, but not E_{KL} ($t=-1.27$, $P=0.22$) or E_P ($t=1.25$, $P=0.23$; Fig. 15). Percent Congruity ($57.8 \pm 16.4\%$) did not change with speed ($t=-0.16$, $P=0.88$), nor did %Recovery ($26.0 \pm 18.1\%$; $t=0.23$, $P=0.82$; Fig. 16).

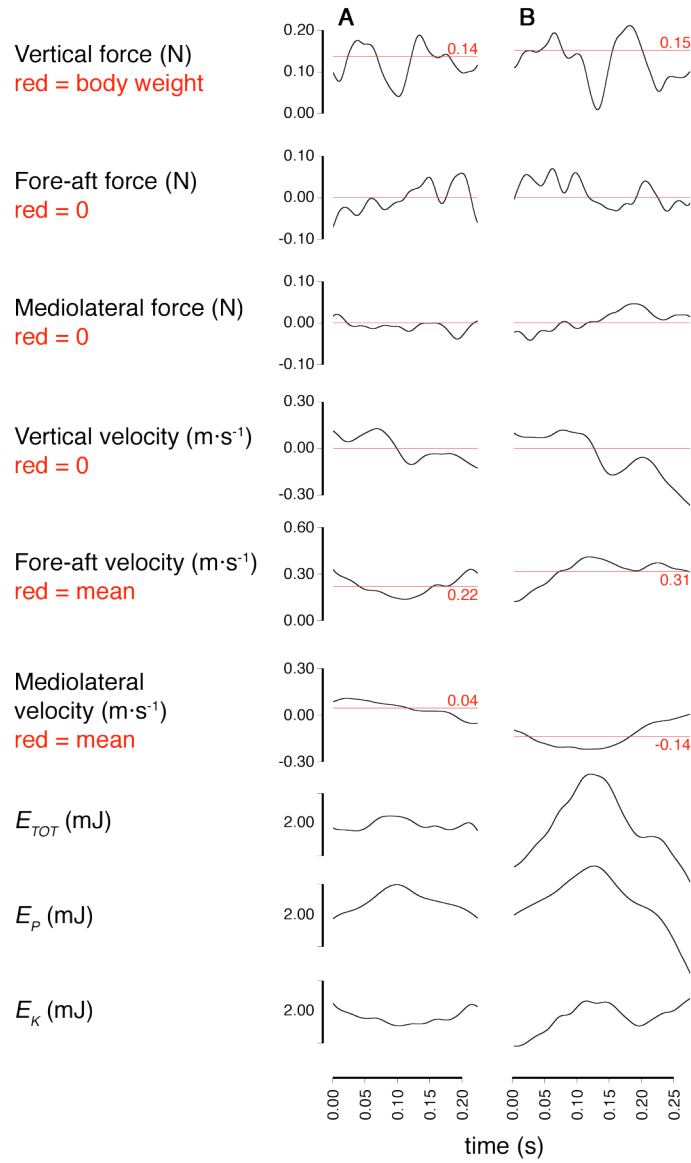


Figure 14: Energetics of two separate stride cycles, left hind footfall to left hind footfall, of *M. tuberculata* performing (a) a kinetic walk-like stride cycle (body mass 14.0 g, speed = 0.27 m·s⁻¹, %Congruity = 19.3%, %Recovery = 59.5%), and (b) a kinetic run-like stride cycle (body mass = 15.5 g, speed = 0.28 m·s⁻¹, %Congruity = 60.0%, %Recovery = 24.0%). Though speed is similar in these two trials, the energetics of the former feature greater pendulum-like changes in E_K and E_p than the latter. Despite such variability in COM energetics from trial to trial, *M. tuberculata* did not transition from a kinetic walk to a kinetic run with increasing speed.

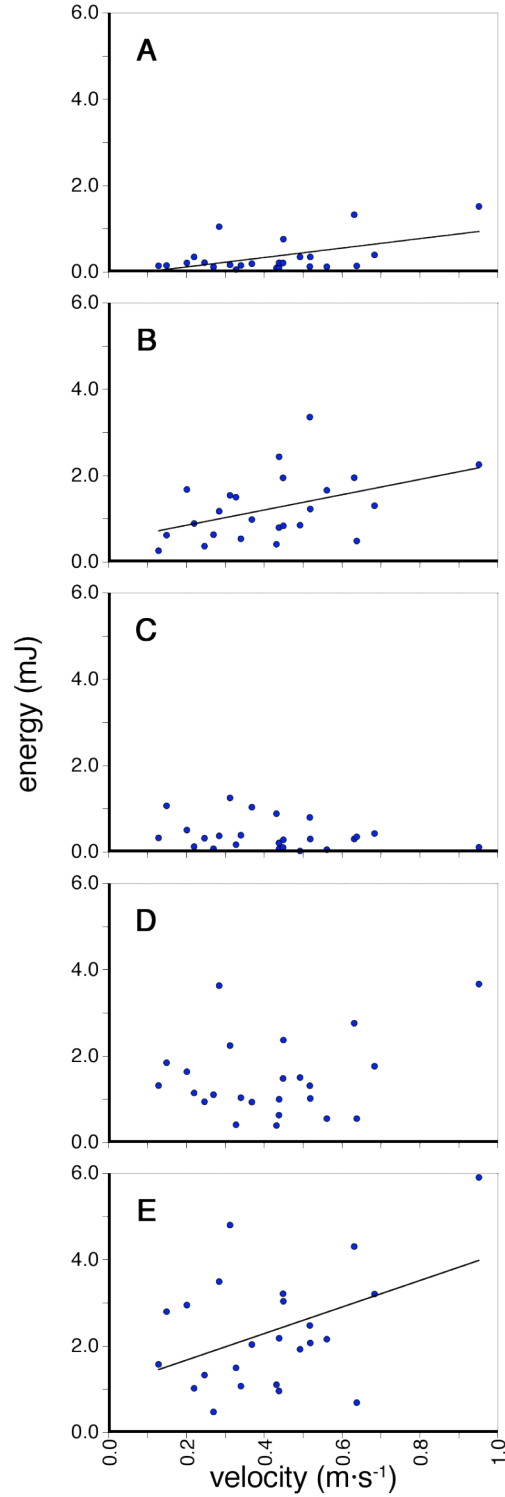


Figure 15: Magnitudes of oscillations in (a) E_{KV} , (b) E_{KF} , (c) E_{KL} , (d) E_P , and (e) E_{TOT} of *M. tuberculata* walking across the force plates at a range of speeds. Bats increased the magnitudes of fore-aft and vertical E_K oscillations with speed, but not of lateral E_K nor of E_P .

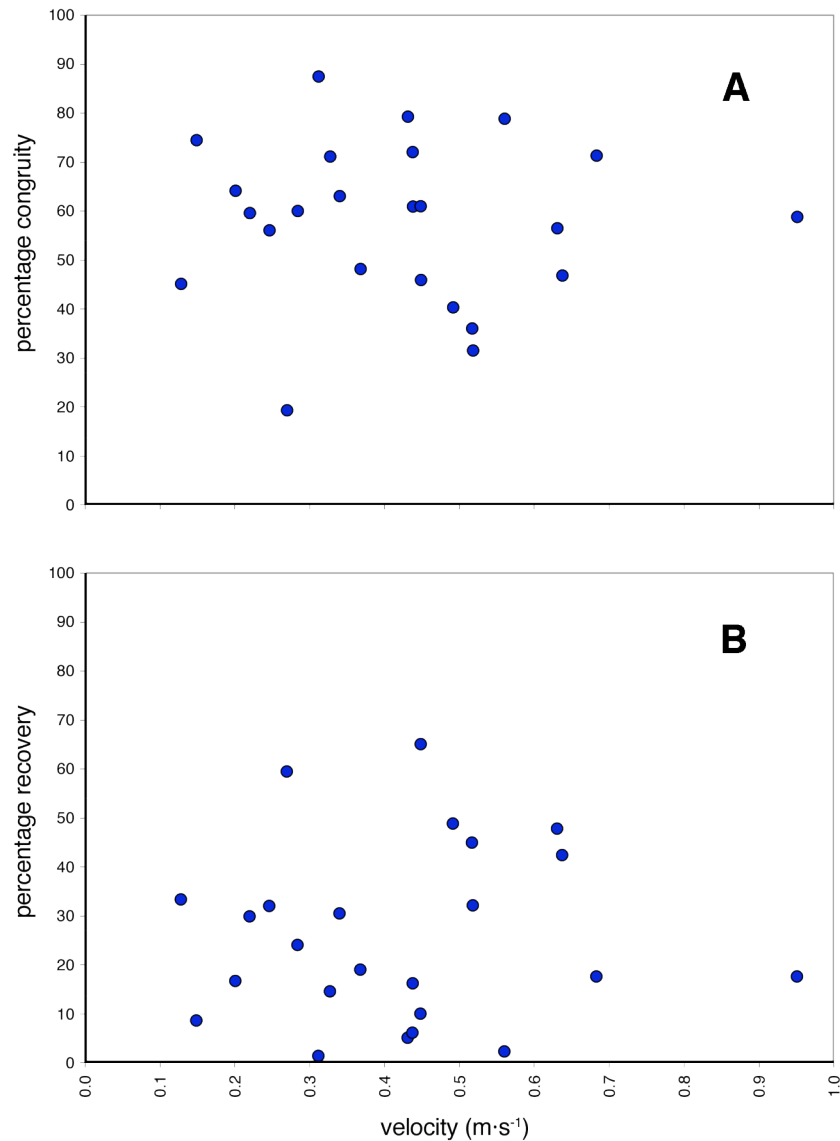


Figure 16: (a) %Congruity and (b) %Recovery and of *M. tuberculata* crossing the force plates at a range of speeds. The considerable variability of values for both these descriptive statistics supports our observation that the patterns of vertical body movement were extremely variable from trial to trial, both on force plates and on the treadmill. A transition from an energetic walk to an energetic run with increased speed would be reflected by an increasing %Congruity and decreasing %Recovery, but neither regression has a slope significantly different from zero.

Discussion

The kinematic walking gaits of both species

We do not find evidence that the ability to fly in these bats prevents them from walking like other tetrapods do. Despite bodies that are highly specialized for flight, both *D. rotundus* and *M. tuberculata* perform lateral sequence walking gaits that are very similar to each other, and to the symmetrical lateral sequence walks known from a broad range of tetrapods, including amphibians, turtles, crocodilians, and the majority of quadrupedal mammals (Hildebrand, 1985; Figs. 17,18). The walking gaits of bats meet two kinematic definitions of walking that are based on the walks of other animals; there is no aerial phase, and the duty factors of forelimbs and hindlimbs are greater than 0.5.

The kinematic walks of *D. rotundus* and *M. tuberculata* are not completely alike, and change differently as speed increases. While both species increase stride frequency with increasing speed, *D. rotundus* keep duty factor somewhat constant in the forelimbs and hindlimbs across speeds. Although *M. tuberculata* follow this pattern with the forelimbs, the duty factor of their hindlimbs decreases with speed. The functional basis of this difference is not clear, but it is interesting that bounding *D. rotundus* decrease duty factor in both forelimbs and hindlimbs as speed increases. In this regard, the lateral-sequence walk of *M. tuberculata* is an intermediate between the walk and bound of *D. rotundus*.

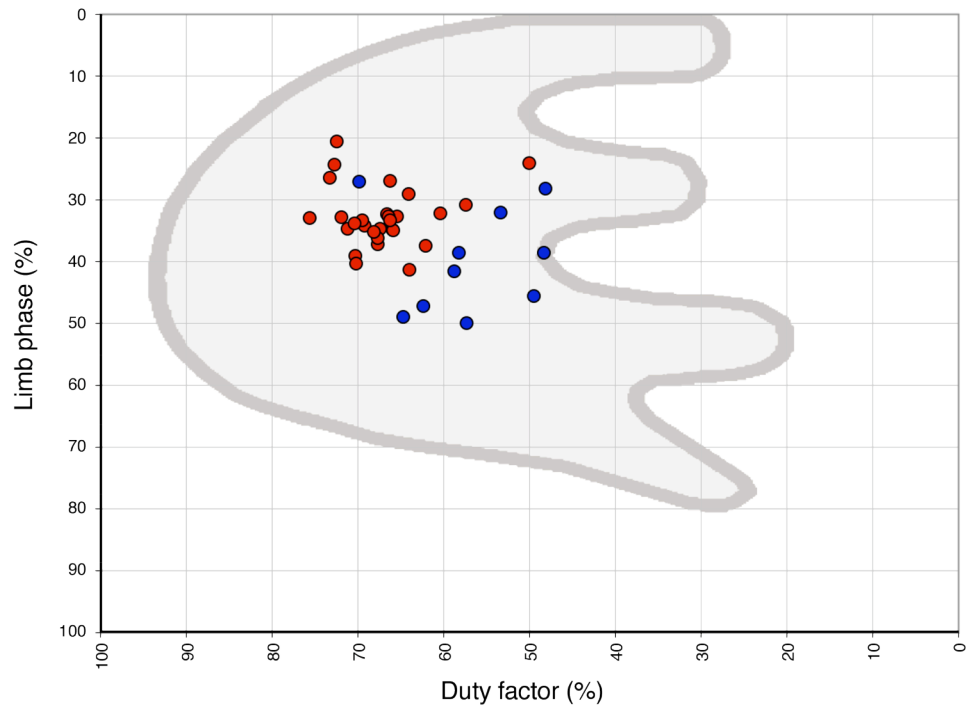


Figure 17: A Hildebrand gait plot for the walking gait of *D. rotundus* (red) and the single gait of *M. tuberculata* (blue). Duty factor is the percent of the stride cycle for which the feet were in contact with the ground, averaged for all four limbs in a stride cycle. Limb phase is the percent of the stride cycle that elapsed between left hindlimb footfall, and left forelimb footfall. The shaded area encloses 1178 symmetrical gait plots from 156 genera of tetrapods (from Hildebrand, 1985).

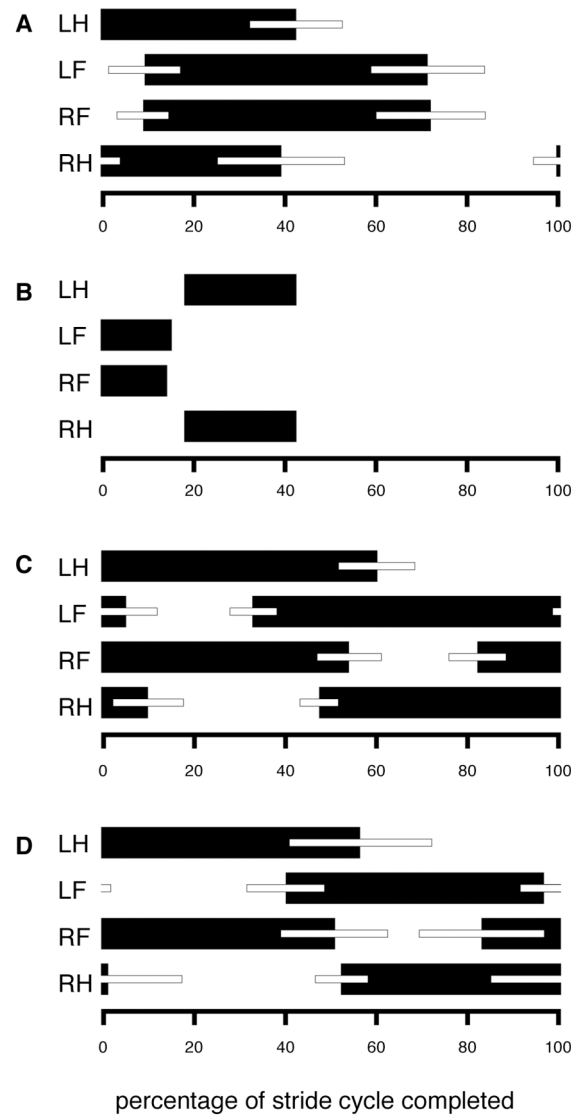


Figure 18: Footfall patterns, beginning and ending with left hind footfall, on the treadmill for (a) bounding *D. rotundus*, (b) a bounding quadrupedal rodent (from Hildebrand, 1985), (c) walking *D. rotundus*, and (d) *M. tuberculata* using their single gait. Solid bars indicate time that a foot is in contact with the ground. Hollow bars represent one standard deviation above and below the mean. F = fore; H = hind; L = left; R = right. Note that the bounding gait of *D. rotundus* is superficially similar to the bounding rodent gait, but with the footfall patterns of the forelimbs and hindlimbs reversed.

The bounding Common Vampire Bat gait

To our knowledge, the bounding vampire bat gait is kinematically distinct from any other tetrapod gait known. Definitions of walking and running based on duty factor are not appropriate descriptors for this gait, since by those definitions the forelimbs of bounding *D. rotundus* walked (duty factor >0.5) while the hindlimbs simultaneously ran (duty factor <0.5). However, since there is an aerial phase, the gait clearly meets one kinematic definition of a run (Riskin and Hermanson, 2005). We call the vampire run a bound, because it is superficially similar to the bounding gaits of several terrestrial mammals, including squirrels, jumping mice, and tree shrews (Hildebrand, 1985; Jenkins, 1974). Both types of bounds are asymmetrical, because the footfalls of the forefoot and hind foot on the same side of the body are unevenly spaced in time (Hildebrand, 1966, 1977, 1980). However, compared with the bounding gaits of terrestrial mammals, the roles of the forelimbs and hindlimbs are reversed in vampire bats. In the bounding gait of vampire bats, the duty factor of the forelimbs is greater than that of the hindlimbs and the aerial phase is initiated by push-off with the forelimbs. In bounding terrestrial mammals the reverse is true (Fig. 8a,b).

The evolution of vampire bat running

We have suggested previously that the bounding vampire bat gait is an independently evolved run (Riskin and Hermanson, 2005). This is supported by the fact that a running gait has not been reported for any bat species other than *D. rotundus*. Even the closely related and quadrupedally agile White-winged Vampire Bat (*Diaemus youngi*) does not bound, even

when placed on the same treadmill as that used in this experiment (DKR, GGC, JWH, personal observations).

We propose that as the mammals that gave rise to bats became adapted to flight, they completely lost the ability to run, and that as *D. rotundus* adapted to their unique blood-feeding niche, they ‘re-invented’ running. Because bats have far more musculature in the forelimbs than in the hindlimbs (Strickler, 1978), the population of bats ancestral to *D. rotundus*, when selected for high-speed terrestrial locomotion, would have a morphology more suitable to the evolution of a wing-powered run than a hindlimb-driven one. That vampire bats independently converged on the bounding gaits of other vertebrates supports the hypothesis that quadrupedal animals are forced to choose from a limited range of possible gaits to achieve stability on the ground (Cartmill et al., 2002; Hildebrand, 1985; Jenkins, 1974).

The kinematically defined walking gaits of *D. rotundus* and *M. tuberculata*, on the other hand, are probably synapomorphic with those of other terrestrial vertebrates. While the complete inability (or refusal) to crawl has been reported for some hipposiderid, mormoopid, phyllostomid, rhinolophid, and natalid bat species (Dietz, 1973; Lawrence, 1969; Riskin et al., 2005; Schutt and Simmons, 2006; Vaughan, 1959), the distant relationships of non-crawling bats to *D. rotundus* and *M. tuberculata* suggest that the ability to walk was retained throughout the evolution of the bats in this study (Jones et al., 2002; Teeling et al., 2003). Indeed, even among some species that do not crawl as adults, the ability to crawl is retained in juveniles (Dietz, 1973).

It is unlikely that the running gait of vampire bats evolved as a way of permitting them to travel long distances, since flight allows animals to travel greater distances per unit energy than the terrestrial gaits do (Alexander, 2005). Also, Hildebrand (1985) noted that the bounding gait of other mammals is energetically inefficient, and is generally used only over short distances. Bats in our study used the bounding gait for <60 s at a time, and demonstrated fatigue after only a few trials, so the gait appears useful for increasing overall speed in short bursts, rather than for metabolic efficiency over long distances. We therefore infer that in nature the gait has significance to short-term behaviours. Specifically, we suggest that the running gait helps *D. rotundus* follow prey animals that flee or move in the middle of a feeding event.

The feeding behaviour of *D. rotundus* prior to the introduction of livestock to their range in the 16th century is unknown. Captive *D. rotundus* are known to take blood from a broad range of vertebrates, including porcupines, armadillos, small rodents, and even snakes (Greenhall, 1988), so it is plausible that some of the wild animals upon which these bats feed might attempt to evade them by running away. Carranza and Campo (1982) once observed *D. rotundus* feeding on a capybara (Rodentia: *Hydrochoerus* sp.) that fled upon being disturbed by researchers. As the capybara ran toward the water, the vampire bat chased after it on the ground without taking flight. Since vampire bats often take some time to locate and prepare a bite area before feeding begins (Greenhall, 1988), locomotory strategies to follow prey that move during a feeding event would have an obvious energetic benefit.

COM energetics of locomotion in the New Zealand Short-tailed Bat

As *M. tuberculata* increased speed, the amount of energy used to accelerate the COM in both the vertical and fore-aft directions increased, while the range of heights through which the COM traveled did not. This suggests that as speed increases, the way in which energy is cycled among potential and kinetic forms changes. However, we did not observe an increase in %Congruity nor a decrease in %Recovery with increasing speed. *M. tuberculata* therefore use a kinetically variable gait that does not transition from a kinetic walk to a kinetic run with increased speed.

The magnitudes of changes in E_K and E_P were similar, suggesting that energy could be exchanged between them in a pendulum-like manner. However, based on its values of %Recovery, the single kinematic gait of *M. tuberculata* is more kinetically run-like than walk-like. Known values of %Recovery in quadrupeds range from as high as 80% in penguins (Griffin and Kram, 2000) to as low as 30-40% in walking frogs, rams, lizards, and giant tortoises (Ahn et al., 2004; Cavagna et al., 1977; Farley and Ko, 1997; Zani et al., 2005), and even less than 5% in opossums (Parchman et al., 2003). The values of %Recovery in this study (ca. 26%) certainly fall in the lower end of this spectrum. The inverted-pendulum mechanism of energy conservation therefore does not appear to be of particular importance to *M. tuberculata* at any speed.

Tradeoffs in the locomotion of bats

In this study we found no evidence of tradeoffs for flight in the terrestrial locomotion of *D. rotundus* or *M. tuberculata*. Their walking gaits fell well within the range of kinematic gaits known for terrestrial quadrupeds,

and though the running gait of *D. rotundus* is unique, there is no evidence that it is any less efficient than the gaits of terrestrial mammals. In fact, Heglund and Taylor (1988) found a correlation between stride frequency and metabolic cost during the locomotion of terrestrial mammals, so the decreased stride frequency of bounding vampire bats compared with similarly sized mice (Riskin and Hermanson, 2005) suggests that vampire bats might even consume less energy while running than other mammals do.

In *D. rotundus* and *M. tuberculata*, evolution from the ancestral condition of diminished crawling ability to their current states of terrestrial agility resulted in kinematic gaits similar to those of other tetrapods. An obvious future research question is to determine whether terrestrial agility has imposed a cost on the ability to fly in these species, since various anatomical features suggest that a tradeoff exists. Bats that are terrestrially agile have greater muscle mass in the pectoral girdle than bats that do not (Strickler, 1978), and *D. rotundus* are known to possess slow-twitch muscle fibres in the pectoralis muscle that are absent in bats that do not crawl well (Hermanson et al., 1993). A cost to terrestrial agility might be associated with the upkeep of muscle fibres, or with some other aspect of morphology, such as hindlimb orientation (Schutt and Simmons, 2006; Simmons, 1994; Vaughan, 1959).

Alternatively, it is possible that no tradeoff between aerial and non-aerial agility exists in bats at all, and that bats are simply absent from terrestrial niches for other reasons, such as competition with other mammals (Daniel, 1979). Indeed, *M. tuberculata* evolved in the absence of terrestrial mammal competitors, and vampire bats occupy a niche that is not occupied

by any other mammal. Furthermore, the wing shapes of neither species suggests a reduced ability to fly compared with other bats (Jones et al., 2003; Norberg and Rayner, 1987; Webb et al., 1998). The presence or absence of a tradeoff would best be tested by measurements of oxygen consumption during flight in bats that move on the ground well and bats that do not. If *D. rotundus* and *M. tuberculata* suffer tradeoffs between these forms of locomotion, we predict a greater rate of oxygen consumption during flight for those species than for bats that avoid the ground most of their lives. With the knowledge from this study that bats move on the ground like other mammals do, such investigations of flight energetics will help us understand how an animal meets the demands of more than one form of locomotion.

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CHAPTER 5: RETROSPECTIVE

Summary

This dissertation has provided a few valuable insights about the way in which bats move on the ground. I have demonstrated that the most widely cited explanation for the poor crawling performance of bats is incorrect; bats are not hindered in their terrestrial performance by the dimensions of the tibia or of the femur. I have shown that two of the most terrestrial bat species, Common Vampire Bats (*D. rotundus*) and New Zealand Short-tailed Bats (*M. tuberculata*) use quadrupedal gaits that are kinematically similar to the lateral sequence walks of other tetrapods. Using kinetic definitions, the walking gait of *M. tuberculata* is more run-like than walk-like, and does not shift from walk-like to run-like with increasing speed. *D. rotundus* also possess a running gait that is unique compared to those of other vertebrates, but converges kinematically on the bounding gait used by small terrestrial mammals, with the roles of the forelimbs and hindlimbs reversed. I propose that this represents the independent evolution of a running gait in bats, wherein the increased musculature of the forelimbs compared with the hindlimbs predisposed bats to evolve a forelimb-driven gait.

Many questions remain. Even with the hindlimb-strength hypothesis ruled out, it remains to be understood why terrestrial locomotion is so uncommon among bats, compared with birds, for example. It is also not clear how the anatomy of some species make them better suited to terrestrial locomotion than other bats are. If such anatomical specializations exist, they could make quadrupedal bats less efficient in aerial locomotion

than are bats that do not perform coordinated terrestrial locomotion. Experiments designed to test for underperformance in flight would be helpful. This system of study shows great promise toward improving our understanding of how bats move on the ground, and how their morphology is shaped by the selective pressures associated with their life histories.

On the use of kinetic gait definitions

In the fourth chapter of this thesis, I made use of summary statistics that are used to describe animal locomotion, including %recovery, %congruity, and various other measurements taken from the force plates as bats walked across them. These are reported widely, and typically thought to be useful for understanding the efficiency of locomotion of animals (see Zani et al., 2005 for a summary). However, some authors have stated recently that such measurements are not so useful at all, and distract researchers from better-reasoned measurements of animal movement (Ruina et al., 2005). Below, I briefly argue that regardless of functional significance, descriptive statistics like %recovery are useful for comparing animals to one another.

In the 1970's Cavagna et al. applied the measurements taken from force plates to the inverted pendulum model of locomotion, reasoning that by measuring movement of the COM, they might perceive how animals use pendulum-like mechanics to save energy while walking. Cavagna and others created descriptive statistics to describe the way in which E_K and E_P of the COM change during locomotion, so that such measurements could be summarized neatly. One obvious advantage of these statistics is that they

provide a means by which the locomotion of different organisms can be compared. Since all animals can be simplified to a point mass with kinetic and gravitational potential energy, any locomotory strategy can be explained in terms of how that point mass moves. While it is difficult to compare the footfall patterns of bipedal, quadrupedal, or hexapodal animals, their COM kinetics are easily compared using this methodology (Cavagna et al., 2002; Kram et al., 1997; Zani et al., 2005).

However, the link between COM kinetics and metabolic efficiency is not understood. In fact, Andy Ruina (personal communication) has passionately argued that %recovery has no meaningful relationship to the kinetics of locomotion at all. It will be interesting to see how the statistics developed by the Cavagna group withstand future testing. It should be clear from the fact that I used them in my own work, that I see value in such descriptive statistics. Specifically, I found %recovery useful for comparing bats to other vertebrates that move on the ground.

Animals from disparate lineages often use similar means of locomotion. For example, swimming by means of flagella can be found among prokaryotes, protists, and even human sperm. Legged locomotion occurs in tetrapods, arthropods, and mollusks. Wheeled locomotion appears not to have evolved in any lineage. Understanding how animals of such broadly differing body types converge in their locomotor patterns suggests constraints, biomechanical, ontogenetic, or otherwise, that cause animals to move in similar fashions. By measuring %recovery, I have published data on the locomotion of *M. tuberculata* that can be incorporated into surveys of animal locomotion. In other words, the very fact that people report %recovery so frequently makes its measurement valuable in other animals.

If a link is someday understood between %recovery and metabolic efficiency, such studies will be all the more valuable, but they are already helpful in understanding animal diversity regardless of their utility for understanding how animals move.

Consider as a parallel, the careful measurement and description of skull shape in bats. It is clear that the sagittal crest provides a larger surface for the origin of *temporalis* muscles than would be possible in its absence, and so its functional contribution to bite force (and feeding ecology) is understood (Freeman, 1984). On the other hand, basisphenoid pits occur in many disparate bat taxa, have no known function, and indeed, may have no adaptive value at all (DeBaeremaeker and Fenton, 2003). To say that one is more important than the other when describing the anatomy of the animal assumes that only information about biomechanical function is useful. However, if the purpose of your skull investigation is understanding phylogenetic relationships, the basisphenoid pits will be far more useful than the height of the sagittal crest; characters that do not correlate strongly with ecology are less driven by convergent evolution than other characters, and thus provide better information about relationships among species. When the information gained from biomechanical investigations is used in other contexts, measurements like %recovery are still useful for comparing species (as I have done), even where the functional significance is unknown.

In this dissertation I mostly limited my discussion of %recovery and %congruity to its utility for comparing bats to other species, but did conclude that “the inverted-pendulum mechanism of energy conservation... ..does not appear to be of particular importance to *M. tuberculata* at any speed.” This sentence is based on the assumption that a body that does not move

like a pendulum cannot save energy like a pendulum does. Based on what we have measured, and how it compares with other animals, I think it is logical to make this assertion. If some day I am proven wrong, that sentence will no longer hold true, but value of the data I have reported on the terrestrial locomotion of *M. tuberculata* will persist.

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