

Walking and running in the red-legged running frog, *Kassina maculata*

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Summary

Although most frog species are specialized for jumping or swimming, *Kassina maculata* (red-legged running frog) primarily uses a third type of locomotion during which the hindlimbs alternate. In the present study, we examined *Kassina*'s distinct locomotory mode to determine whether these frogs walk or run and how their gait may change with speed. We used multiple methods to distinguish between terrestrial gaits: the existence or absence of an aerial phase, duty factor, relative footfall patterns and the mechanics of the animal's center of mass (COM). To measure kinematic and kinetic variables, we recorded digital video as the animals moved over a miniature force platform ($N=12$ individuals). With respect to footfall patterns, the frogs used a single gait and walked at all speeds examined. Duty factor always exceeded 0.59. Based on COM mechanics, however, the frogs used both walking and running gaits. At slower speeds, the fluctuations in the horizontal kinetic energy (E_k) and gravitational potential

energy (E_p) of the COM were largely out of phase, indicating a vaulting or walking gait. In most of the trials, *Kassina* used a combined gait at intermediate speeds, unlike cursorial animals with distinct gait transitions. This combined gait, much like a mammalian gallop, exhibited the mechanics of both vaulting and bouncing gaits. At faster speeds, the E_k and E_p of *Kassina*'s COM were more in phase, indicating the use of a bouncing or running gait. Depending on the definition used to distinguish between walking and running, *Kassina* either only used a walking gait at all speeds or used a walking gait at slower speeds but then switched to a running gait as speed increased.

Movies available on-line.

Key words: walking, running, gait, locomotion, biomechanics, *Kassina maculata*, anuran.

Introduction

The kinematics of limb movement and/or the mechanics of the animal's center of mass (COM) generally distinguish walking from running gaits during terrestrial locomotion. Historically, variables easily obtained from video or film images, such as the presence or lack of an aerial phase, the fraction of a cycle during which a single foot is on the ground (duty factor) and relative footfall patterns have allowed researchers to determine the gaits used by animals (Muybridge, 1957; Alexander, 1977; Hildebrand, 1985). More recently, however, the mechanical energy fluctuations of an animal's COM during locomotion are often used to distinguish walking from running gaits (Cavagna et al., 1977). This last method is particularly useful for animals with more than two legs and those that may not achieve an aerial phase, even at their fastest speeds (McMahon et al., 1987; Blickhan and Full, 1987; Full and Tu, 1990; Farley and Ko, 1997). Alexander (1989) has argued that a discontinuous change in one or more mechanical or kinematic parameters important to an animal's locomotion defines a gait transition.

Classically, the definitions of walking *versus* running gaits have been based on duty factor, or the fraction of the stride during which the animal's limb is in contact with the ground.

Duty factors of >0.5 provide a kinematic characterization of walking gaits. By contrast, duty factors of <0.5 typically characterize running gaits (Alexander, 1977, 1989; Hildebrand, 1985), during which a reference limb of an animal contacts the ground for $<50\%$ of the stride cycle. This method of gait determination allows the evaluation of locomotion with a single video camera and has been used to examine gaits in mammals, amphibians and birds (Muybridge, 1957; Alexander, 1977; Hildebrand, 1985; Ashley-Ross, 1994; Gatesy, 1999; Reilly and Biknevicius, 2003; Hutchinson et al., 2003).

In mechanical terms, differences in the patterns of the kinetic (E_k) and potential energy (E_p) fluctuations of an animal's COM during locomotion have also been used to distinguish walking and running gaits. By examining the E_k and E_p fluctuations of the COM of an animal, walking can be modeled as a rolling egg, with the limbs and body functioning like an inverted pendulum, whereas running can be modeled as a bouncing ball, with the limbs and body functioning like a simple spring-mass system (Cavagna et al., 1977). During the stance phase of walking, the animal's COM vaults over each limb following an upward then downward trajectory.

Correspondingly, the fluctuations in E_k and E_p of its COM are largely out of phase (Cavagna et al., 1977). By contrast, during running, the COM generally loses height in phase with the animal's deceleration during the first half of limb support. The COM then rises in phase with the animal's acceleration during the second half of limb support. This downward then upward trajectory of the COM is accomplished with the compression and re-extension of the spring-like legs during the stance phase of running, which results in the largely in-phase fluctuations of the E_k and E_p of the animal's COM. Similar to a bouncing ball, energy can be conserved through elastic storage and release in the muscles, tendons and joint ligaments of the animal's legs (Cavagna et al., 1977). A force platform is used to measure the instantaneous ground reaction forces of the whole animal during terrestrial locomotion. From these measurements, the movements of the animal's COM are determined to provide a mechanical basis for the principles of energy exchange that distinguish terrestrial walking and running gaits. This mechanical method is most useful when examining animals with varying leg number, leg orientation, body shape or skeletal type and has been used to identify the walking and running gaits of a variety of mammals, birds, crabs, insects and lizards (Cavagna et al., 1977; Heglund et al., 1982; Blickhan and Full, 1987; Full and Tu, 1991; Muir et al., 1996; Farley and Ko, 1997; Parchman et al., 2003).

Walking and running gaits are rarely studied in frogs, mainly because past research has focused on their swimming and jumping abilities (Calow and Alexander, 1973; Zug, 1978; Emerson, 1979; Marsh, 1994; Lutz and Rome, 1994; Kamel et al., 1996; Peters et al., 1996; Peplowski and Marsh, 1997; Gillis and Biewener, 2000). Although most frog species are specialized for hopping and swimming, *Kassina maculata* primarily uses a third locomotor mode: a gait during which the animal's left and right fore- and hindlimbs alternate over a broad range of speeds. Some frogs also use gaits with alternating hindlimbs during swimming (Nauwelaerts and Aerts, 2002) and when walking at very slow speeds (Gray, 1968; Walton and Anderson, 1988; Anderson et al., 1991). However, *Kassina* uses this walking/running-type gait as its primary mode of locomotion at all speeds on land (Fig. 1; see Results).

In the present study, we examine the kinematics and mechanics of *Kassina*'s locomotion to determine whether these frogs use a walking or running gait (or both) and how speed influences their gait. *Kassina*'s alternating limb gait does not appear to change as they increase speed (Fig. 1). Unlike gait changes that are clear and easily discernible in mammalian quadrupeds, *Kassina*'s gait at fast speeds appears simply to be a faster version of its gait at slow speeds. More typical patterns in mammals show drastic changes in the footfall patterns accompanying the gait changes from walking to trotting to galloping with increasing speed (Alexander, 1977; Biewener, 2003). We test the general hypothesis that *Kassina* does not change gait with speed. Based on the uniform visual appearance of *Kassina*'s locomotion at slow and fast speeds (Fig. 1), we hypothesize that *Kassina* uses a vaulting or walking gait at all speeds. Based on previous studies that show

animals can use a bouncing, running gait without aerial phases, however, an alternative hypothesis is that *Kassina* uses a bouncing or running gait at all speeds (McMahon et al., 1987; Full and Tu, 1990).

Materials and methods

Animals

Data were collected from 17 frogs (*Kassina maculata* D., mass 8.33 ± 1.72 g) with a mean snout-vent length (SVL) of 0.044 ± 0.004 m. The animals were obtained from commercial suppliers (markmlucas.com; Coral Springs, FL, USA; www.cyberaqua-net.com; Exotic Jungles, Inc., Oakland Park, FL, USA). All animals were fed crickets dusted with calcium and vitamin powder and had free access to water. The animal room was maintained at $20\text{--}22^\circ\text{C}$ with a reverse 12 h:12 h L:D photoperiod. All experiments were performed at room temperature. All experiments were performed in accordance with the Animal Care and Use Guidelines of Harvard University.

Kinematic variables

Speed, duty factor and limb phase of the animals were determined from the video recordings. High-speed digital video data were recorded at 125 frames s^{-1} (Redlake PCI-500 MotionScope) as the animals moved over the force platform. The video camera was positioned to obtain a direct lateral view of the animal, but, additionally, a mirror placed at an angle allowed a simultaneous dorsal view so that all four limbs were visible in the video recordings (Fig. 1).

Speed

The mean speed of each trial was calculated from the digital video sequences by dividing the length of the force platform (0.12 m) by the time taken by the animal to cross the platform using the animal's eye movement as a reference. With the exception of speeds obtained from treadmill trials (Fig. 2), all data presented were obtained from force platform trials.

Treadmill data were obtained to determine the upper boundary of *Kassina*'s locomotory speeds and to determine whether the speeds recorded during the force platform trials were indicative of the maximal, steady-state speeds of the animals. Therefore, the only trials recorded and analyzed on the treadmill were those at faster speeds. The treadmill (0.21 m \times 0.60 m working space) was much larger than the animal in order to avoid constraining the animal within a confined area. Although *Kassina* tended to move intermittently, the animals often moved at relatively constant or steady-state speeds during the bursts, staying in one position on the moving treadmill belt. The speeds for these constant-speed bursts on the treadmill were compared with the speeds recorded when the animals moved over the force platform (Fig. 2).

Duty factor

Duty factor was the main kinematic parameter used to

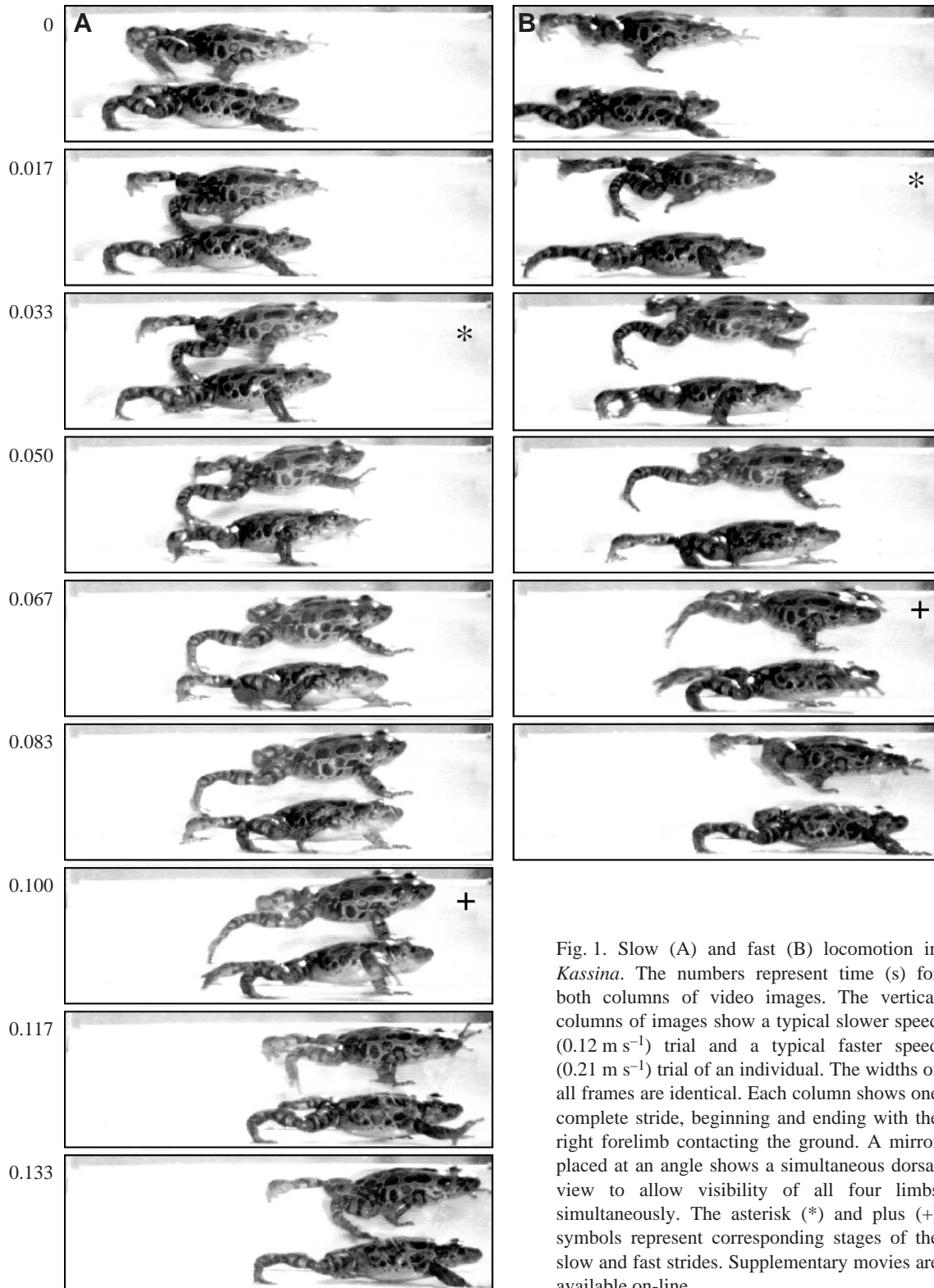


Fig. 1. Slow (A) and fast (B) locomotion in *Kassina*. The numbers represent time (s) for both columns of video images. The vertical columns of images show a typical slower speed (0.12 m s^{-1}) trial and a typical faster speed (0.21 m s^{-1}) trial of an individual. The widths of all frames are identical. Each column shows one complete stride, beginning and ending with the right forelimb contacting the ground. A mirror placed at an angle shows a simultaneous dorsal view to allow visibility of all four limbs simultaneously. The asterisk (*) and plus (+) symbols represent corresponding stages of the slow and fast strides. Supplementary movies are available on-line.

distinguish between walking and running gaits. For each force platform trial, duty factor was calculated as the percentage of the stride cycle during which one reference limb was on the ground. According to Hildebrand's model of symmetrical gaits (Hildebrand, 1985), if a single reference

limb contacted the ground for more than 0.50 of the cycle, then the animal used a walking gait. By contrast, if a single limb contacted the ground for less than 0.50 of the stride cycle, then the animal used a running gait. For the present study, the duty factor measurements were averaged between

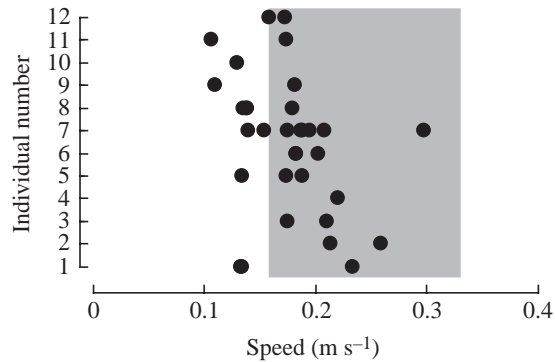


Fig. 2. Speed range observed in the trials used for the present study. The points represent the speeds measured as the frogs moved across the force platform. The shaded region represents the range of speeds observed when the animals ran on a treadmill. Only faster runs were accepted for the treadmill trials. Therefore, the shaded region indicates the upper range of the steady-state speeds attained by the animals in the laboratory.

the right fore- and hindlimbs of a trial, resulting in a representative duty factor for that trial. Duty factor measurements did not differ between fore- and hindlimbs ($P=0.82$; paired t -test).

Limb phase

Diagonal limb (DL) and same-side limb (SSL) phases were calculated as the percentage difference between the times at which the diagonal-side or same-side limbs came into contact with the ground during a stride. For DL phases, the front left and hind right limbs were used for analysis. For SSL phases, we analyzed the side during which the forelimbs contacted the ground first during the stride.

Mechanics of the center of mass

Miniature force platform

To determine the kinetic (E_k) and potential energy (E_p) fluctuations of the animal's center of mass (COM), force platform and digital video data were obtained simultaneously as the animals ($N=12$; mass= 8.38 ± 1.55 g; $SVL=0.044\pm 0.004$ m) moved across a custom-built miniature force platform (Heglund, 1981; Biewener and Full, 1992). A 2.12 m-long track was constructed with three wooden walls and one Plexiglas wall, through which the animals were filmed. The miniature force platform (0.12 m long \times 0.06 m wide) was positioned midway along the length of the running track and set flush to the running surface. The animals moved across the force platform into a darkened cardboard box placed approximately 3 cm from the other end of the force platform. We attempted to collect multiple trials from each animal at varying speeds. However, the animals mostly moved to escape gentle prodding, with many of the fastest and slowest trials tending to include very large accelerations or decelerations. The criteria we used to determine acceptable trials included: (1) the animal completed an entire stride while on the force platform, (2) the relative magnitudes of the E_k and E_p were

within a single order of magnitude of each other and (3) the net horizontal change in velocity was less than 50% of the average velocity across the force platform. Change in velocity, determined from the horizontal force recordings for each trial, was calculated by dividing the difference between the final and initial horizontal velocities by their mean and then multiplying this fraction by 100. Although a $\pm 50\%$ change in velocity is a generous criterion for steady-state locomotion, the nine trials for which the change in velocity was between 25% and 50% showed patterns of kinematics and COM energy changes much like those trials with less than 25% changes in velocity ($P=0.22$ – 0.96 ; unpaired t -tests).

The force platform consisted of a rectangular (0.12 m \times 0.06 m), 2 mm-thick panel of honeycomb aluminum bonded to two brass beams that were supported over an aluminum base. The front and rear brass beams were machined with three independent, single spring blade elements on either side, allowing vertical, horizontal (fore–aft), and medio-lateral forces to be recorded separately. For the purposes of this study, only the vertical and horizontal components of the ground reaction forces were resolved, because the lateral forces generated by the animal were too small to be measured by the force platform. Since lateral forces have been recorded to be less than 5% of the fore–aft horizontal forces in sprawled quadrupeds that laterally undulate during locomotion (Farley and Ko, 1997), omitting the lateral forces would be unlikely to influence the conclusions of the present study. Forces were measured using a pair of single-element, metal foil strain gauges (type FLA-1-11; Tokyo Sokki Kenkyujo Co., Tokyo, Japan) bonded to both sides of each of the 12 spring blades (three spring blades for each of the four corners of the platform). Cross-talk between vertical and horizontal outputs did not exceed 3.1%. Loads in the range of those generated by the animal (0.02–0.2 N) produced a linear response with a maximum variation across the platform of 3.4% ($r^2=0.99$ for vertical and horizontal directions). The sensitivity of the channel outputs in the vertical direction was 0.56 N V^{-1} and the horizontal direction was 0.63 N V^{-1} . The natural, unloaded frequency of the platform equaled 125 Hz. Raw force signals were amplified through Vishay conditioning bridge amplifiers (model 2120; Measurement Group, Don Mills, Ontario, Canada) and collected using data-acquisition software at 5 kHz (Axoscope 8.0; Axon Instruments, Inc., Foster City, CA, USA) on a PC. The data were filtered using a recursive, lowpass Butterworth filter with a cut-off frequency of 25 Hz. The video recordings were post-triggered and synchronized with the force recordings for each trial.

Energy calculations

The vertical and horizontal forces recorded over a complete stride were used to calculate the mechanical energies of the COM of each animal as it traveled across the miniature force platform (Cavagna, 1975). These forces were first integrated to obtain the instantaneous vertical and horizontal velocities. Whereas the integration constant in the vertical direction was zero, the mean speed of the animal was used as the integration

constant to calculate changes in horizontal velocity. To ensure correct calculations, the horizontal velocities calculated from force platform data were compared with the velocities determined from video data. The instantaneous horizontal and vertical velocities were then used to calculate the instantaneous vertical kinetic energy (E_{kv}) and horizontal kinetic energy (E_{kh}) using the formula: kinetic energy = $0.5Mv^2$, where M is the mass of the animal and v is its velocity. The sum of these energies determined the instantaneous total kinetic energy (E_k). An additional integration of the instantaneous vertical velocity gave the instantaneous vertical displacement (h) of the COM to calculate changes in the gravitational potential energy ($E_p = Mgh$, where M is the mass and g represents acceleration due to gravity) of the animal. The total mechanical energy of the COM of the animal at each instant was obtained by adding E_k and E_p . Percentage change in velocity was calculated by dividing the difference between the final horizontal velocity and initial horizontal velocity by the mean horizontal velocity for the trial and then multiplying this fraction by 100.

Phase shift

The phase shift between E_k and E_p was the main mechanical determinant used to distinguish between walking and running gaits. Typically, a 180° phase shift between the E_k and E_p of the COM has defined a walking gait (Cavagna et al., 1977; Farley and Ko, 1997), and a 0° phase shift of the COM energies has defined a running gait (Cavagna et al., 1977; Full and Tu, 1990). For the present study, trials during which the phase shift approximated 180° (or $>135^\circ$) were classified as walks, and trials during which the phase shift approximated 0° (or $<45^\circ$) were classified as runs. The mechanical energy phase shift was determined by dividing the time between the absolute minima of E_k and E_p by the time of a complete stride and multiplying it by 360° .

Percentage recovery

%Recovery, defined as the energy exchanged between E_k and E_p , has been used to evaluate the magnitude of mechanical energy saved by this energy exchange of the COM based on an inverted pendulum model of walking gaits (Cavagna et al., 1976). Because of changes in the relative phases of E_k and E_p during walking (out-of-phase) versus running (in-phase), %Recovery would be expected to be high during walking and low during running. Following Cavagna et al. (1977), %Recovery was calculated as:

$$\% \text{Recovery} = \frac{(\sum \Delta E_k + \sum \Delta E_p - \sum \Delta E_{\text{com}}) \times 100}{(\sum \Delta E_k + \sum \Delta E_p)},$$

where $\sum \Delta E_k$ is the sum of the positive increments in kinetic energy, $\sum \Delta E_p$ is the sum of the positive increments in gravitational potential energy, and $\sum \Delta E_{\text{com}}$ is the sum of the positive increments in total mechanical energy.

For the present study, we did not use %Recovery to classify walks versus runs. Instead, we examined %Recovery only as a correlate of gait, relying on the phase relationship between

the E_k and E_p of the animal's COM as the mechanical determinant of a gait (Full and Tu, 1990; Farley and Ko, 1997). In trials for which the relative magnitudes of the fluctuations in E_k and E_p of the COM were similar (within one order of magnitude), the %Recovery and the phase shift would be expected to positively correlate. A low %Recovery would accompany an in-phase or near-zero phase shift, while a high %Recovery would accompany an out-of-phase or near- 180° phase shift.

Percentage congruity

For the present study, %Congruity was defined as the proportion of the cycle during which the E_k and E_p of the COM changed *similarly* in direction, recognizing that these two mechanical energies were never completely in- or out-of-phase. We adopted %Congruity to provide an additional quantitative approach for assessing how the relative timing of the fluctuations in E_k and E_p correspond to walking versus running gaits. Ideally, %Congruity would be 0% for a walking trial and 100% for a running trial. Lower values of %Congruity indicated a gait that was more like a walk, and higher values of %Congruity indicated a gait that was more like a run. In determining %Congruity for each trial, the instantaneous rate of change of the energy was calculated by differentiating E_k and E_p with respect to time during a stride. When the product of the instantaneous changes in E_k and E_p was greater than zero, the two energies were congruent. Conversely, when the product of the instantaneous changes in E_k and E_p was equal to or less than zero, the energies were incongruent. %Congruity was calculated as the sum of the portions of the cycle during which the two energies changed in similar directions (i.e. when the product of the rates of energy change was greater than zero) divided by the cycle time and multiplied by 100. Although %Congruity neglected the relative magnitudes of the fluctuations in E_k and E_p of COM, it quantifies the relative changes in E_k and E_p throughout the entire stride cycle.

Statistics

All reported values represent means \pm s.d. If more than one trial was obtained for an individual, the data were averaged to represent that individual to avoid pseudo-replication. Comparisons were tested with unpaired *t*-tests. Differences were considered to be statistically significant when $P < 0.05$. Linear regressions, however, were obtained from pooled data ($n=32$) because three trials or fewer were obtained from 10–12 individuals. The linear regressions obtained from pooled data did not result in different conclusions from regressions of averaged data, for which a single data point or a single regression line represented an individual.

Results

Speed

The horizontal speeds (v) of the animals as they crossed the force platform ranged from 0.10 m s^{-1} to 0.30 m s^{-1} (mean = $0.17 \pm 0.03 \text{ m s}^{-1}$; $N=12$ individuals), for which the

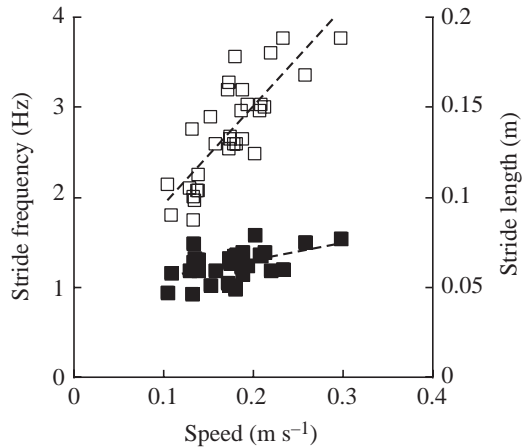


Fig. 3. Stride frequency and stride length as a function of speed. Both stride frequency (open symbols) and stride length (filled symbols) increased with speed, but to different degrees. The animals primarily moved faster by increasing stride frequency.

normalized speeds were in the range of 2.4–6.8 body lengths s^{-1} ($BL s^{-1}$). One to eight trials were obtained from each of the 12 animals, yielding a total of 32 trials examined in this study. The speeds of the animals as they moved on the treadmill ranged from $0.16 m s^{-1}$ to $0.33 m s^{-1}$ (mean = $0.21 \pm 0.04 m s^{-1}$; $N=9$ individuals), demonstrating a large overlap between the faster speeds obtained over the force platform and those obtained on the treadmill (shaded region in Fig. 2).

Kassina mainly increased speed by increasing stride frequency (Fig. 3). As the speed of the animals increased threefold, stride frequency approximately doubled from 1.8 Hz to 3.8 Hz (stride frequency = $11.0v + 0.84$; $r^2=0.67$; $P<0.0001$). The additional increase in speed was achieved by a more modest increase in stride length from 0.05 m to 0.08 m (stride length = $0.097v + 0.05$; $r^2=0.24$; $P=0.004$).

Kinematics – footfall patterns

Duty factor

The duty factors of the animals locomoting over the force platform indicated that the frogs used only a walking gait (Fig. 4). Although duty factor (averaged between fore- and hindlimbs) never measured less than 0.5, it decreased with increasing speed (duty factor = $-1.03v + 0.88$; $r^2=0.62$; $P<0.05$) and ranged from 0.77 to 0.59 (mean = 0.69 ± 0.05 ; $N=12$ individuals) over the speed range recorded. On the treadmill, the two fastest trials observed ($0.28 m s^{-1}$ and $0.33 m s^{-1}$) also exhibited duty factors indicative of walking gaits (0.67 and 0.69, respectively).

Limb phase

Diagonal limb (DL) phase and same-side limb (SSL) phase were both nearly independent of forward speed (Fig. 5). DL phase ranged from 4.8% to 16.1% (mean = $11.5 \pm 2.4\%$; $N=12$ individuals) and was independent of speed (DL phase = $-9.71v + 13.26$; $r^2=0.02$; $P=0.44$). SSL phase ranged from 31.4% to 48.9% (mean = $38.4 \pm 4.2\%$; $N=12$ individuals)

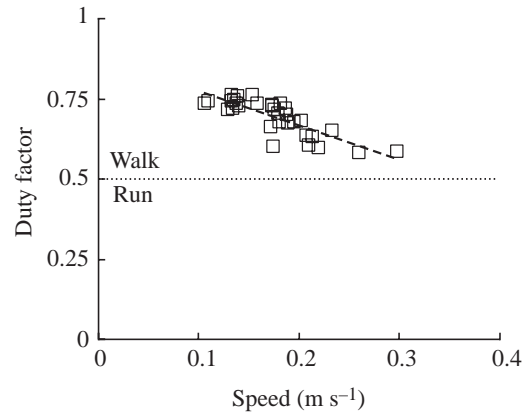


Fig. 4. Duty factor versus forward speed. As the animals moved faster, the duty factor, or the portion of the stride cycle during which a single limb was on the ground, decreased but was never less than 0.5.

and varied slightly with speed (DL phase = $52.39v + 28.78$; $r^2=0.25$; $P=0.0035$). A DL phase of 0% and an SSL phase of 50% would indicate a trot-like running gait. The limb phases observed here, however, showed that these frogs used a gait that was slightly offset from a trot, during which the diagonal limbs contacted the ground at slightly different times. Although the limb phase data failed to discriminate whether the animals ‘walked’ or ‘ran’, they did suggest that the gait used did not change with speed.

Mechanical energies of the COM

Walking frogs

Four of the 32 trials showed that three animals used a mechanical walking gait, during which the phase shift between E_k and E_p exceeded 135° (Figs 6A, 7A). In these trials, the phase shift between the minima of E_k and E_p ranged from 147° to 200° (mean = $170 \pm 26^\circ$; $N=3$ individuals). These trials during which the phase shift approximated 180° indicate that the minimum E_k occurred simultaneously with the maximum E_p

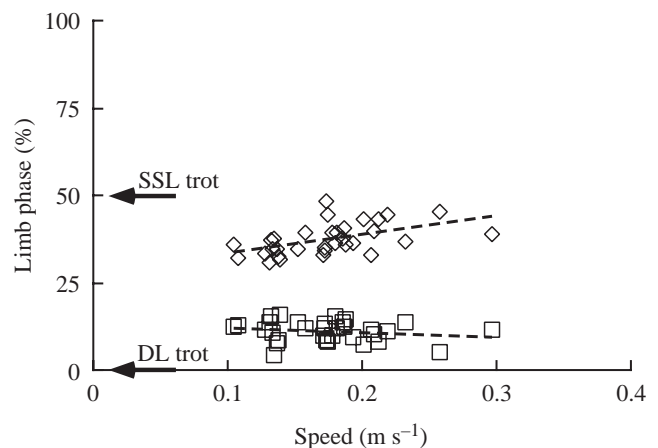


Fig. 5. Footfall patterns as a function of speed. Both diagonal limb (DL; squares) phase and same-side limb (SSL; diamonds) phase remained constant or varied little with speed.

(e.g. Fig. 6A), consistent with the pattern of energy exchange expected for an inverted pendulum. Although speed did not strictly determine gait, walking tended to occur at slower speeds in *Kassina* ($<2 \text{ m s}^{-1}$).

Running frogs

In 21 of the 32 trials examined, the phase shift between E_k and E_p was less than 45° (Figs 6B, 7A), indicating that nine animals used a bouncing or mechanical running gait. In these trials, the phase shift between the minima of the mechanical energies ranged from 3° to 33° (mean= $12 \pm 9^\circ$; $N=9$ individuals; $P<0.05$ when compared with phase shift during walking), indicating that the E_k fluctuations correlated closely with the fluctuations in E_p (Fig. 6B). As with walking, speed did not strictly determine gait in *Kassina*. However, the fastest trials observed consisted of only running trials.

An intermediate gait

In the remaining seven trials, the phase shift between E_k and E_p was between 45° and 135° , indicating that these seven animals did not use a well-defined vaulting or bouncing gait (Fig. 7A). In these trials, the phase shift between the minima of the mechanical energies ranged from 47° to 131° (mean= $88 \pm 31^\circ$; $N=7$ individuals; $P<0.05$ when compared with phase shift during walking and running), indicating that the E_k fluctuations were neither consistently in-phase nor out-of-phase with the fluctuations in E_p (Fig. 7A).

Percentage recovery

%Recovery generally decreased with increasing speed in *Kassina* (Fig. 7B). %Recovery, on average, was higher in walking trials (range, 21.9–44.2%; mean= $32.0 \pm 10.9\%$; $N=3$) than in running trials (range, 3.9–28.0%; mean= $15.4 \pm 6.3\%$; $N=9$; $P<0.05$), but considerable overlap existed in terms of both speed and %Recovery values. Moreover, %Recovery for the trials during which the animals used an intermediate gait did not differ from %Recovery during walking and running trials (range, 6.6–39.1%; mean= $25.8 \pm 11.8\%$; $N=7$; $P>0.05$).

Percentage congruity

%Congruity increased linearly with forward speed (%Congruity= $200.5v+19.5$; $r^2=0.34$; $P<0.05$; Fig. 7C) and matched closely the (inverse) pattern observed for %Recovery (Fig. 7B). When using phase shift to differentiate walking from running, %Congruity was lower during walking (range, 32.0–44.6%; mean= $36.5 \pm 5.2\%$ of the stride; $N=3$) than during running (range, 46.5–75.1%; mean= $62.4 \pm 7.9\%$; $N=9$; $P<0.05$). In general, the mechanical energies during all walking and most of the intermediate gait trials showed opposing directional changes in E_k and E_p of the animal's COM, indicating a vault-like gait. By contrast, higher values of %Congruity indicated similar directional changes in E_k and E_p of an animal's COM

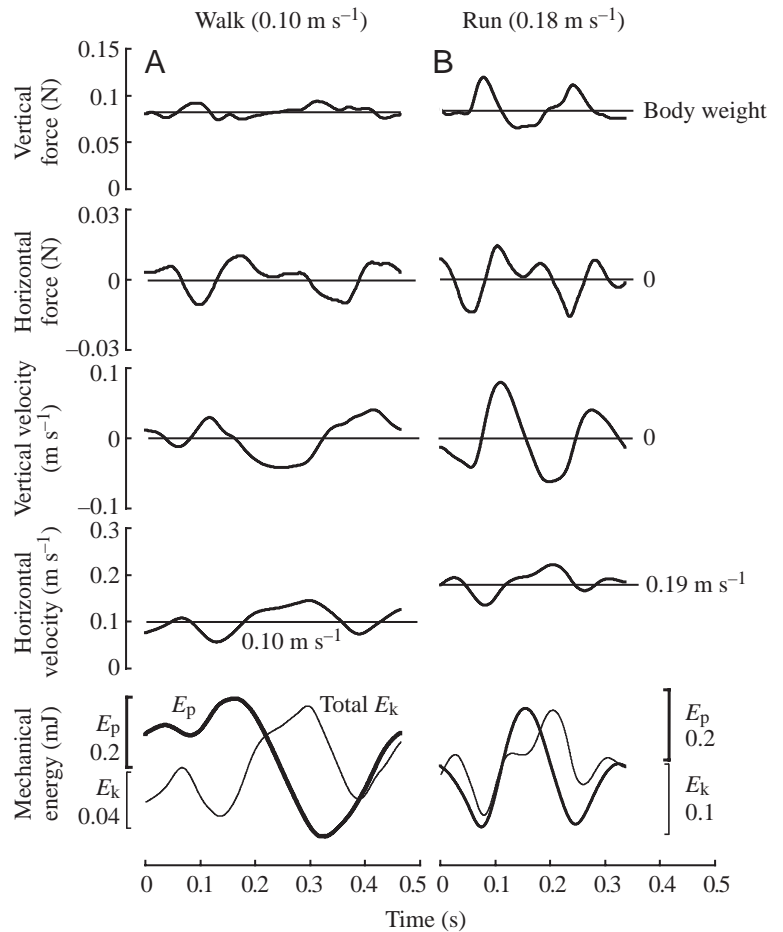


Fig. 6. Representative ground forces, center of mass (COM) velocities, and COM mechanical energies during a 'mechanical walk' (A) and a 'mechanical run' (B). For each trial, a single complete stride is shown. For the walking trial, animal weight= 0.083 N ; speed= 0.10 m s^{-1} ; stride frequency= 2.2 Hz ; phase shift= 147° ; recovery= 24.3% ; duty factor= 0.74 ; same-side limb (SSL) phase= 36.2% ; diagonal limb (DL) phase= 13.0% ; congruity= 39.9% ; external mechanical power= 0.11 W kg^{-1} . For the running trial, animal weight= 0.085 N ; horizontal velocity= 0.19 m s^{-1} ; stride frequency= 3.0 Hz ; phase shift= 3.4° ; recovery= 14.5% ; duty factor= 0.73 ; SSL phase= 38.1% ; DL phase= 14.3% ; congruity= 61.1% ; external mechanical power= 0.21 W kg^{-1} . E_k , horizontal kinetic energy; E_p , gravitational potential energy.

and a bouncing gait (Fig. 7C). However, many of the trials, including those defined as walking and running using the phase shift definition, exhibited congruities near 50%, characterizing a gait that likely combined the vaulting and bouncing mechanisms within a single stride. Through a large range of intermediate speeds, the animal's COM often exhibited vaulting mechanics in addition to bouncing mechanics (Fig. 8), during which E_k and E_p fluctuated out-of-phase or in-phase during different periods of the stride cycle.

Discussion

Depending on the definition used to distinguish between walking and running, our results show that *Kassina* either uses

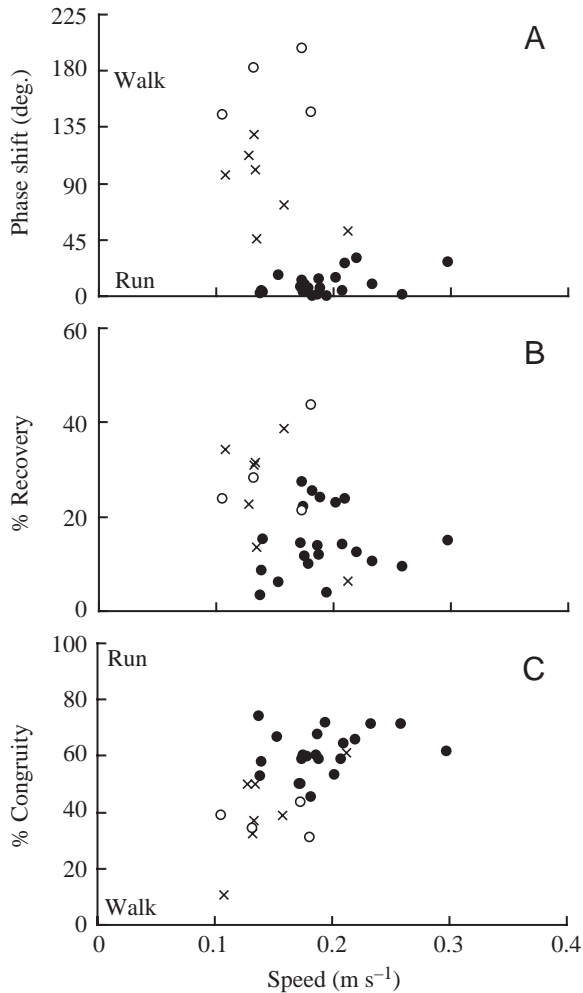


Fig. 7. Phase shift (A), %Recovery (B) and %Congruity (C) as a function of forward speed. Walking (open circles) was considered to occur when the phase shift between horizontal kinetic energy (E_k) and gravitational potential energy (E_p) of the center of mass (COM) was out-of-phase or near 180° ; running (filled circles) was considered to occur when the phase shift between the E_k and E_p of the COM was in-phase or near 0° . Those trials during which the phase shift was neither in- nor out-of-phase were categorized into an intermediate gait (crosses). (B) %Recovery generally decreased with speed. (C) %Congruity increased with forward speed and generally showed a similar separation as %Recovery with respect to walking and running mechanical trials.

only a walking gait at all speeds or uses different gaits by walking at slower speeds, running at faster speeds and combining walking and running gait mechanics at most intermediate speeds. Based solely on kinematic determinants of gait, footfall patterns indicate that over a threefold increase in speed *Kassina* only uses a walking gait, in support of our hypothesis (Figs 4, 5). In contrast to our kinematic hypothesis and findings, the mechanical behavior of *Kassina*'s COM suggests that these frogs walk at slower speeds, use a 'galloping-like' gait at intermediate speeds and run at faster speeds (Figs 6–8).

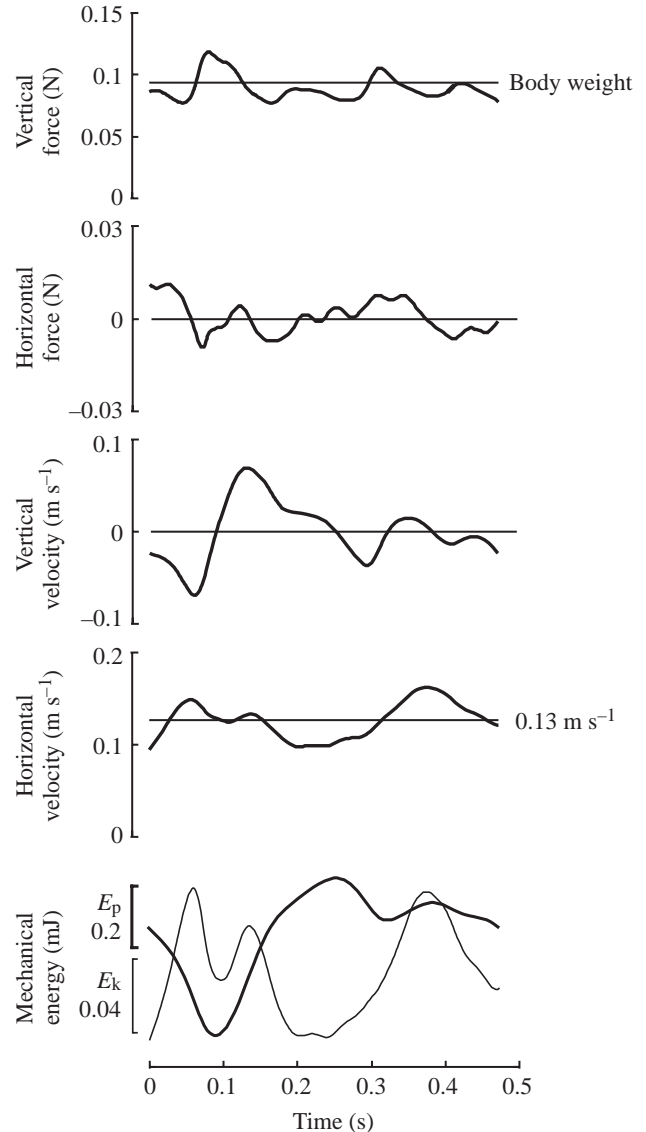


Fig. 8. Representative ground forces, center of mass (COM) velocities, and COM mechanical energies during a gait that combined vaulting and bouncing mechanics. Similar to a mammalian gallop, *Kassina* often used a gait that vaulted for half of the stride and bounced for half of the stride, resulting in an intermediate %Congruity. A single complete stride is shown, for which animal weight=0.094 N, horizontal velocity=0.13 m s⁻¹, stride frequency=2.1 Hz, phase shift=114°, recovery=23.1%, duty factor=0.72, same-side limb (SSL) phase=33.9%, diagonal limb (DL) phase=11.9%, congruity=50.5%, and external mechanical power=0.13 W kg⁻¹. E_k , horizontal kinetic energy; E_p , gravitational potential energy.

Kinematic evaluation of gait

From an evaluation of *Kassina*'s footfall patterns, we found that these frogs use only a walking gait (Fig. 9). At very slow speeds, *Kassina* attains static stability with duty factors that approximate 0.75 of the stride (Fig. 4), as has been observed in other frogs and quadrupeds (Gray, 1968; Alexander, 1977). With increasing speed, the time that each limb contacts the

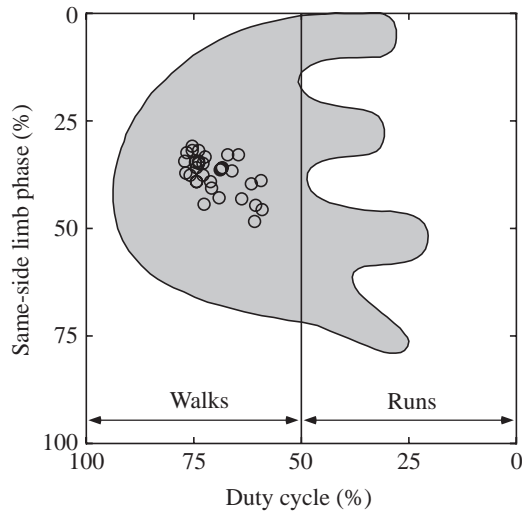


Fig. 9. Hildebrand plot. Using solely kinematics, the footfall patterns indicate that *Kassina* only walked at all speeds. Duty cycle always exceeded 50%. Adapted from Hildebrand (1985).

ground decreases, but each limb always remains in contact with the ground for greater than half the stride (duty factor >0.59) over the observed speeds and conditions. Even at the fastest speeds, *Kassina* never exhibits an aerial phase. Although animals can use a bouncing-type of gait, such as a trot, without an aerial phase, the footfall pattern of *Kassina* shows that the diagonal limbs contact the ground at slightly offset times (Fig. 5). Albeit 'trot-like', this footfall pattern contrasts the trotting patterns typically seen in mammals and some reptiles (Hildebrand, 1985; Farley and Ko, 1997). Based on their footfall patterns, we conclude that *Kassina* walks at all speeds.

Mechanical evaluation of gait

Mechanical energy fluctuations

Although *Kassina*'s gait does not change kinematically with speed, the E_k and E_p of *Kassina*'s COM during locomotion suggest that these frogs not only use both walking and running gaits but also a gait mechanically equivalent to a mammalian gallop. *Kassina* uses the same two basic mechanisms observed previously in mammals, birds, reptiles and arthropods (Cavagna et al., 1977; Heglund et al., 1982; Blickhan and Full, 1987; Full and Tu, 1990; Farley and Ko, 1997). At slower speeds, *Kassina* occasionally uses a vaulting or walking gait when moving, as indicated by the E_k and E_p of its COM changing out-of-phase and incongruously (Figs 6A, 7). At faster speeds, *Kassina* occasionally uses a running gait, much like a spring-mass system or bouncing ball, during which the E_k and E_p of its COM change mostly in-phase during a stride (Figs 6B, 7). More often, at many of the speeds observed in the lab, *Kassina* employs a gait that combines the mechanics of a vaulting gait and a bouncing gait, during which %Congruity between E_k and E_p of the animal's COM approximates 50%. This combined gait is mechanically

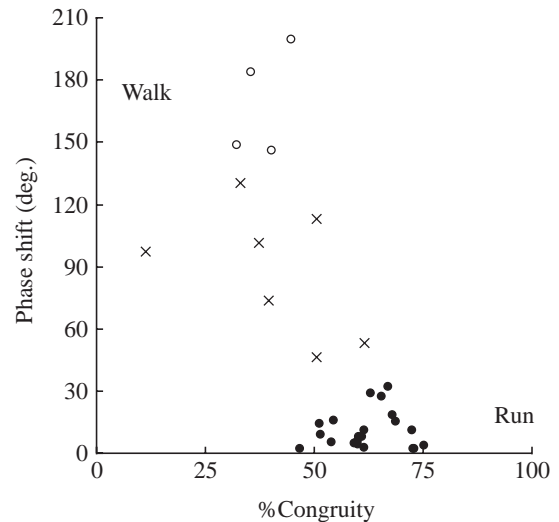


Fig. 10. Mechanical determinants of walking and running. For the present study, phase shifts near 180° (greater than 135°) defined walking (open circles), phase shifts between 45° and 135° defined the intermediate gait (crosses), and phase shifts near 0° (less than 45°) defined running (filled circles). The two variables generally agreed (upper left and lower right quadrants), as demonstrated by their inverse relationship.

similar to a mammalian gallop at slow speeds (Fig. 8; Cavagna et al., 1977).

In the present study, we use two criteria to examine the mechanical distinctions between walking and running (Figs 7, 10). The main criterion, the phase shift between the minima of E_k and E_p , has been used to distinguish walking from running in insects, reptiles and mammals (Fig. 7A; Full and Tu, 1990; Farley and Ko, 1997; Parchman et al., 2003). Additionally, we calculate a secondary criterion, %Congruity, to examine more completely the relative fluctuations in E_k and E_p of the COM throughout the entire stride cycle (Fig. 7C). %Congruity, or the proportion of the cycle during which the E_k and E_p of the COM change *similarly* in direction, indicates whether the COM behaves mechanically more like a vaulting system or more like a bouncing system. Unlike bipeds, the existence of fore- and hindlegs in quadrupeds may complicate the movements of the COM. For example, elephants locomote at faster speeds by vaulting with their forelimbs, during which their forelimbs move upwards then downwards during stance, and bouncing with their hindlimbs, which move downwards then upwards during the stance phase of fast locomotion (Hutchinson et al., 2003). As would be expected for elephants, %Congruity during walking or running in *Kassina* does not show ideal vaulting or ideal bouncing behavior. Instead, mechanical energies of its COM are 36% congruent for walking frogs and 62% congruent for running frogs (Fig. 7C). %Congruity, thus, may be an additional, useful measure of gait type when the COM energies do not fluctuate like those of ideal point-mass systems, which probably would not occur in any animal system.

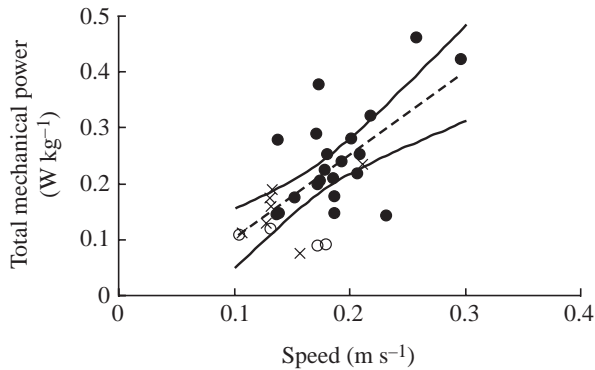


Fig. 11. Mass-specific external mechanical power required to lift and accelerate the center of mass as a function of forward speed. Mechanical power increased with speed during terrestrial locomotion in *Kassina* (walking – open circles; intermediate gait – crosses; running – filled circles; $\text{power} = 1.5v - 0.05$; $r^2 = 0.45$; $P < 0.0001$; solid lines represent 95% confidence intervals). The slope of the linear regression (broken line) represents the mechanical work required to move the center of mass (COM) by 1 m ($1.5 \text{ J kg}^{-1} \text{ m}^{-1}$).

Mechanical work to lift and accelerate the COM

As the product of positive work per stride and stride frequency, the total external mechanical power required to lift and accelerate the COM increases with speed (Fig. 11). On a mass-specific basis, *Kassina* expends 1.5 J kg^{-1} to travel 1 m, which is similar to that required for *Coleonyx* and *Eumeces* lizards ($1.5 \text{ J kg}^{-1} \text{ m}^{-1}$; Farley and Ko, 1997). This value also falls within the range observed in other walking and running animals, such as birds, mammals, crabs and insects ($0.13\text{--}1.7 \text{ J kg}^{-1} \text{ m}^{-1}$; Heglund et al., 1982; Blickhan and Full, 1987; Full and Tu, 1990), and excludes the internal mechanical work needed to move the limbs relative to the COM. This consistent range of mass-specific mechanical energy values required to move a given distance in such diverse group of animals suggests very similar and general principles underlying the mechanism of legged, terrestrial locomotion (Heglund et al., 1982; Full, 1989).

Resolving differences between terrestrial gait definitions

The discrepancy between gait definitions based on kinematics versus mechanics is not new. Early locomotion studies showed that mammals use a vaulting gait with high duty factors (walk) at slow speeds and then switch to a bouncing gait with low duty factors (run or trot) at faster speeds (Fig. 9; Muybridge, 1957; Cavagna et al., 1977; Hildebrand, 1985). Several subsequent studies, however, have shown this distinction between gaits to be less ubiquitous. For example, McMahon et al. (1987) showed that humans who run with bent legs (i.e. 'groucho running') use a mechanical bouncing gait, even without an aerial phase. Insects (Full and Tu, 1990, 1991) and opossums (Parchman et al., 2003) also use bouncing gaits based on their COM mechanics without including an aerial phase. By contrast, lizards employ a trotting footfall pattern even though they use a walking gait based on their COM mechanics (Farley and Ko, 1997). Although the basic patterns

and principles of walking and running gaits were defined decades ago, exceptions to the basic rules continue to surface, which may simply reflect the earlier bias towards the study of cursorial mammals and birds.

Gait transitions

Regardless of its locomotor gait or the definition used to determine its locomotor gait, *Kassina* does not appear to exhibit a clear terrestrial gait transition. The walk–run transition typically occurs at a distinct speed for a given species (Hoyt and Taylor, 1981; Heglund and Taylor, 1988; Farley and Taylor, 1991; Kram et al., 1997). In mammalian quadrupeds, this transition is often associated with a distinct change in the slope of the relationship between stride frequency and speed (Heglund and Taylor, 1988). In *Kassina*, however, stride frequency and duty factor vary linearly and continuously with speed (Figs 3, 4). Neither parameter exhibits a change in slope or a curvilinear relationship as observed in quadrupedal mammals and avian bipeds (Gatesy and Biewener, 1991; Gatesy, 1999). Furthermore, diagonal limb phase does not depend on speed and exhibits no change in slope. Mechanically, *Kassina* uses a walking gait at slower speeds and a running gait at faster speeds, but there also exists a large range of intermediate speeds over which *Kassina* uses a gait that combines the mechanics of vaulting and bouncing gaits (Figs 7C, 8). This gait, which approaches 50% congruity, is mechanically similar to the slow gallop of quadrupedal mammals (Cavagna et al., 1977). Although *Kassina* clearly does not gallop like cursorial mammals, these frogs may be utilizing a locomotory mode between walking and running gaits. Unlike cursorial animals, the gait transition between walking and running occurs over a broader range of speeds in frogs and lizards (Fig. 7; Farley and Ko, 1997).

Although *Kassina* primarily utilizes a gait with an alternating hindlimb sequence of footfall patterns, it also hops and swims like many other anuran species. With increasing speed, *Kassina* tends to walk/run faster rather than resort to jumping. By contrast, Fowler's toads (*Bufo woodhousei fowleri*) change gaits from walking to hopping by gradually decreasing the relative frequency of walking steps to hops with increasing speed (Anderson et al., 1991). Anderson et al. (1991) suggest that toads cannot maintain moderate and high speeds of locomotion by only walking because their shorter forelimbs and longer hindlimbs prevent them from increasing stride length sufficiently. Likewise, *Kassina* also increases stride frequency more than stride length to move faster, but without resorting to jumping at faster speeds (Fig. 3). Even though Fowler's toads are 2.5× larger than *Kassina* in body size, *Kassina* is able to achieve much faster speeds ($0.1\text{--}0.3 \text{ m s}^{-1}$) than the toads ($<0.1 \text{ m s}^{-1}$; Anderson et al., 1991) using an alternating hindlimb sequence gait.

Conclusions

Although *Kassina maculata* appears to use only one terrestrial gait based on footfall patterns, it uses two mechanical mechanisms to move at different speeds and, thereby, may be

considered to be using at least two gaits. Like other limbed animals, *Kassina* uses a strut-like mechanism to walk and a bouncing, spring–mass-like mechanism to run. Unlike many other terrestrial mammals and birds that walk and run, *Kassina* never achieves an aerial phase and its footfall patterns do not change substantially with speed. At slower speeds, during a mechanically defined walk, the E_k and E_p of *Kassina*'s COM are 170° out-of-phase and 37% congruent. At a broad range of intermediate speeds, *Kassina* combines the mechanics of both walking and running gaits. Finally, at faster speeds, *Kassina* uses a bouncing or mechanical running gait during which the E_k and E_p of its COM are largely in-phase (mean 12° phase difference) and 62% congruent. During running, energy is conserved by its storage and release in the elastic elements of the animal's musculoskeletal system with each 'bounce' or stride (Cavagna et al., 1977). Therefore, *Kassina*'s switch to a bouncing gait at faster speeds may be energetically favorable compared with hopping, as hopping in toads has been found to be energetically more costly compared with running in other terrestrial animals (Anderson et al., 1991). The kinematic and mechanical patterns of *Kassina* are similar to those observed in a variety of other terrestrial animals such as lizards, opossums, cockroaches and 'groucho-running' humans. Although the basic COM mechanisms identified for walking and running gaits apply to a diversity of terrestrial animals, the kinematics associated with them can be quite varied and may not reflect distinct gait transitions in terms of limb movement patterns. Thus, *Kassina*'s common name, the red-legged running frog, is apt in terms of its locomotor body mechanics but not in terms of its limb kinematics.

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