Running over rough terrain: guinea fowl maintain dynamic stability despite a large unexpected change in substrate height

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Summary

In the natural world, animals must routinely negotiate varied and unpredictable terrain. Yet, we know little about the locomotor strategies used by animals to accomplish this while maintaining dynamic stability. In this paper, we perturb the running of guinea fowl with an unexpected drop in substrate height (ΔH). The drop is camouflaged to remove any visual cue about the upcoming change in terrain that would allow an anticipatory response. To maintain stability upon a sudden drop in substrate height and prevent a fall, the bird must compensate by dissipating energy or converting it to another form. The aim of this paper is to investigate the control strategies used by birds in this task. In particular, we assess the extent to which guinea fowl maintain body weight support and conservative spring-like body dynamics in the perturbed step. This will yield insight into how animals integrate mechanics and control to maintain dynamic stability in the face of real-world perturbations. Our results show that, despite altered body dynamics and a great deal of variability in the response, guinea fowl are quite successful in maintaining dynamic stability, as they stumbled only once (without falling) in the 19 unexpected perturbations. In contrast, when the birds could see the upcoming drop in terrain, they stumbled in 4 of 20 trials (20%, falling twice), and came to a complete stop in an additional 6 cases (30%). The bird's response to the unexpected perturbation fell into three general categories: (1) conversion of vertical energy ($E_V = E_P + E_{Kv}$) to horizontal kinetic energy (E_{Kh}), (2) absorption of E_V through negative muscular work ($-\Delta E_{com}$), or (3) converting E_P to vertical kinetic energy (E_{Kv}), effectively continuing the ballistic path of the animal's center of mass (COM) from the prior aerial phase. However, the mechanics that distinguish these categories actually occur along a continuum with varying degrees of body weight support and actuation by the limb, related to the magnitude and direction of the ground reaction force (GRF) impulse, respectively. In most cases, the muscles of the limb either produced or absorbed energy during the response, as indicated by net changes in COM energy (E_{com}). The limb likely begins stance in a more retracted, extended position due to the 26 ms delay in ground contact relative to that anticipated by the bird. This could explain the diminished decelerating force during the first half of stance and the exchange between E_P and E_K during stance as the body vaults over the limb. The varying degree of weight support and energy absorption in the perturbed step suggests that variation in the initial limb configuration leads to different intrinsic dynamics and reflex action. Future investigation into the limb and muscle mechanics underlying these responses could yield further insight into the control mechanisms that allow such robust dynamic stability of running in the face of large, unexpected perturbations.

Introduction

In the complex natural environment, legged terrestrial animals must negotiate variable terrain, start, stop, turn, jump, land and recover from unexpected perturbations. Unsteady locomotion is likely to present quite different mechanical requirements and selective pressures from steady-state locomotion. Consequently, the mechanics of unsteady terrestrial locomotion have a great importance for the biology of terrestrial organisms.

Research on legged locomotor mechanics over the past few decades have revealed some of the fundamental mechanisms that animals employ during steady forward locomotion. Through analysis of the energetic fluctuations of the body center of mass (COM), Cavagna and colleagues (Cavagna,

1975; Cavagna et al., 1976, 1977) discovered that during walking, the kinetic energy (E_K) and gravitational potential energy (E_P) of the body cycle out of phase, whereas during running, E_K and E_P cycle in phase. These observations led to the description of simple mechanical models for walking and running that describe how animals can use energy-exchange mechanisms to improve the efficiency of locomotion. In walking, an inverted pendulum mechanism allows E_K to be stored and recovered as E_P during stance, whereas during running, an elastic recoil mechanism provides storage and subsequent recovery of energy in the elastic structures of the limb (e.g. Alexander, 1984; Alexander and Bennet-Clark, 1977; Biewener, 2003; Biewener and Baudinette, 1995; Daley and Biewener, 2003). These energy-exchange mechanisms, the inverted pendulum and elastic recoil, may help minimize the energetic cost of steady locomotion.

Based on these observations, researchers have used a simple spring-mass model to describe the stance phase dynamics of steady forward running (Blickhan, 1989; McMahon, 1985; McMahon and Cheng, 1990). This model, consisting of a point mass attached to a massless, linear 'leg spring', can accurately predict many aspects of stance dynamics during steady running given the appropriate combination of initial velocity, leg length, limb contact angle and limb stiffness (k_{leg}).

In the natural environment animals must maintain dynamic stability of running in the face of unexpected perturbations. To accomplish this, animals must adjust limb parameters as necessary to avoid stumbling or falling and return the system to a steady periodic motion. Work on humans hopping in place and running forward has demonstrated that changes in k_{leg} can help maintain similar COM motions over surfaces of varying compliance (Ferris and Farley, 1997; Ferris et al., 1998, 1999; Kerdok et al., 2002). The stability of mass-spring running can be further improved by adjusting leg contact angle (Seyfarth et al., 2002), which is accomplished automatically if the leg retracts during late swing phase (Seyfarth et al., 2003). These studies demonstrate simple control strategies that animals might employ to maintain stability using conservative mass-spring dynamics. However, the extent to which animals use such mechanisms when running over uneven terrain, and the relative importance of each, is not yet known.

Adjustment of leg-spring stiffness has often been emphasized as a control strategy during running. Whereas changes in k_{leg} may be sufficient to adjust to running over surfaces of varying compliance but high resilience, running in the natural environment often involves interaction with surfaces that are distinctly non-elastic. Recently, Moritz and Farley (2003) used a damped (viscous) surface to perturb hopping dynamics in humans and found that they compensated for the energy dissipated by the surface through net energy production by the limb, to preserve (apparent) Hookean, spring-like motion of the COM through limb actuation. This provides further evidence that maintenance of the total energy and trajectory of the COM is a primary control task during bouncing gaits, but demonstrates that this task can be accomplished through non-spring-like action of the limb during unsteady movement.

Feed-forward anticipatory control, intrinsic mechanical effects and reflex feedback all play important roles in the control of locomotion. The relative importance of these control mechanisms and the way they are integrated with locomotor mechanics certainly depends on context, including: the sensory information available, prior experience of the animal, speed of movement and type of perturbation. By studying the response of the system to controlled perturbations we can further understand this complex interplay between mechanics and control and predict when the system will follow conservative mass-spring dynamics, when it will deviate from this, and whether the system will remain stable. Although most research on the mechanics of stabilization in terrestrial locomotion has focused on informed and trained human subjects, a few studies have investigated the mechanical response to unexpected perturbations. When humans or monkeys land on a platform after passing through a false surface, muscle activity is coordinated to the anticipated time of landing on the false surface; however, reflexes may also contribute to the recovery (Dyhre-Poulsen and Laursen, 1984; McDonagh and Duncan, 2002). Intrinsic mechanics of the musculoskeletal system allow cockroaches to stabilize their COM trajectory within one step after a lateral impulsive perturbation (Jindrich and Full, 2002). Similarly, humans exhibit changes in k_{leg} before changes in muscle activity when they are surprised by a surface of different stiffness during hopping (Moritz et al., 2004). These studies highlight the importance of understanding how anticipatory control, intrinsic mechanical changes and reflex feedback are coordinated to provide locomotor stability.

Yet, at present we know very little about control strategies used by animals to recover from the types of perturbations they face while running in the natural world. To this end, we perturb the running of guinea fowl *Numida meleagris* L. by subjecting them to an unexpected drop in substrate height (ΔH) that is camouflaged to remove any visual cue about the upcoming change in terrain. The dynamic response of the body immediately following this unanticipated perturbation will provide insight into how animals integrate mechanics and control to achieve a simple bouncing gait with robust dynamic stability.

To understand the control strategies used by this avian biped during running, we assess the extent to which guinea fowl maintain weight support and conservative spring-like body dynamics immediately following the perturbation. Dynamic stability requires avoiding falls and returning to steady periodic motion. To accomplish this following a sudden drop in substrate height, the bird must dissipate energy, convert it to another form, or perform some combination of both. A conservative mass-spring system does not allow a net change in total mechanical energy (E_{com}); the sum of the gravitational potential energy (E_P) and kinetic energies in the fore–aft and vertical directions (E_{Kh} and E_{Kv}, respectively) remains constant. If a perturbation results in a change in one type of mechanical energy, it must be redistributed to another. In

reality a gradation of mechanical responses could occur; thus, it is conceptually useful to consider three hypothetical mechanical extremes. In theory, the bird could compensate entirely for the perturbation by adjusting leg length, contact angle and k_{leg} appropriately to maintain a steady spring-like trajectory. This requires preventing change in any individual components (E_P, E_{Kv}, E_{Kh}) of the total mechanical energy (E_{com}) over the course of the step, and would imply that very rapid control mechanisms (intrinsic mechanical changes or rapid reflexes) are sufficient to completely stabilize the system within one step. Alternatively, and more likely, the body could fall some fraction of the ΔH. In this case the mechanical response depends on the control strategies used by the animal. The resulting ΔE_P can be converted to total kinetic energy E_{Ktot}, increasing the animal's velocity, or alternatively, absorbed through negative muscle work yielding a net energy loss ($-\Delta E_{com}$). If the bird maintains conservative mass-spring running dynamics during unexpected perturbations, any loss in E_P resulting from the perturbation will be converted to E_{Ktot}, resulting in an increased velocity at the end of the perturbed step.

Our second aim is to compare the mechanical response between unexpected *vs* expected perturbations in which the guinea fowl is allowed to see the upcoming change in substrate height. This may provide further insight into the relative importance of intrinsic mechanical properties of the limb and proprioceptive feedback *vs* anticipatory control when visual information is available.

Materials and methods
Animals

We obtained five adult guinea fowl *Numida meleagris* L., body mass=1.95±0.28 kg, 21±1 cm standing hip height (mean ± s.e.m.), from a local breeder and clipped the primary feathers to prevent them from flying. The Harvard Institutional Animal Care and Use Committee approved all procedures. The birds were trained to run steadily and build stamina on a motorized treadmill (Proform, Logan, UT, USA, model PFTL 08040, belt 0.4 m wide, 1.0 m long) for 1–2 weeks and subsequently trained to run steadily across the 8 m long runway used in the experiments. Most birds became accustomed to the runway after 1 or 2 days of training and ran steadily across it at a preferred speed around 3 m s^{-1}. To allow visualization of limb segments, we plucked the bird's feathers to above the hip while it was under anesthesia delivered through a mask (isoflurane, 3% induction, 1–2% maintenance). The joint centers of rotation were found by palpation and marked with high contrast ink.

Experimental procedures

Running trials were conducted on an 8 m×0.4 m runway using a 0.6 m×0.4 m Kistler force plate (model 9281A, Amherst, NY, USA) placed at the midway point. The side walls in the middle 1.8 m were constructed of 6 mm Plexiglass® to allow lateral view high-speed digital video recording from both sides. During 'Control' trials (C; see Movie 1 in supplementary material), the bird ran steadily across the level runway. In 'Unexpected Drop' trials (U; see Movies 2–4 in supplementary material), the runway was elevated relative to the force plate at the midway point, to create a drop in substrate height (ΔH=8.5 cm) that was disguised by tissue paper pulled and held tightly across the gap by white tape that matched the white runway surface (Fig. 1). The U trials were randomized to prevent habituation to the experimental set-up by placing a white 6 mm thick board over the drop between U trials and running the bird several times along a level runway. We conducted no more than 2 or 3 U trials on a given recording day, randomized among 15–20 level trials. When multiple recording sessions were conducted, they were not on consecutive days. Finally, at the end of the last recording day, we conducted 'Visible Drop' trials (V; see Movie 5 in supplementary material), in which the bird encountered the same ΔH as in U trials, but was allowed to see
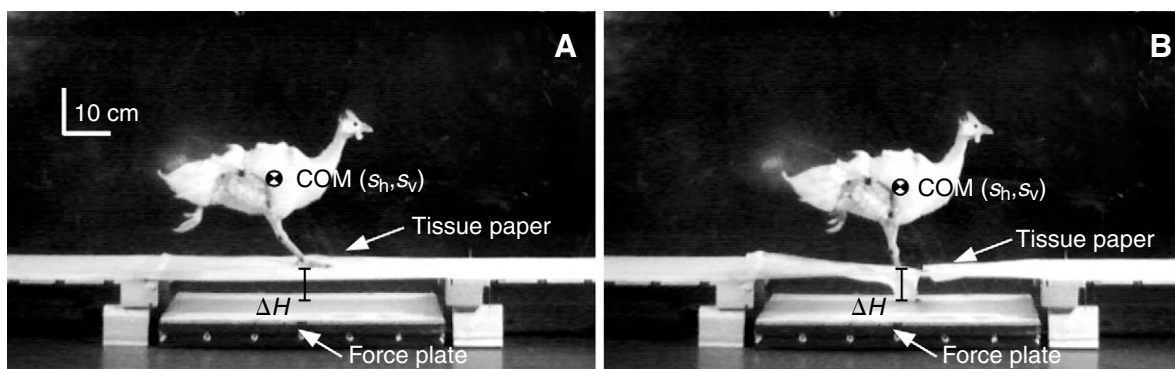

Fig. 1. Still frames of a guinea fowl during an unexpected perturbation. A 0.6 m long force plate placed at the midpoint of an 8 m long runway rested 8.5 cm below the runway surface. White tissue paper pulled tightly across the resulting gap and secured with white masking tape created the appearance of a uniform surface. The velocity and position of the bird's COM through time (moving from frame A to frame B) were calculated through integration of the measured ground reaction forces and used to calculate total COM energy, as described in Materials and methods.

the upcoming change. The birds did not flap or noticeably use their wings when they encountered either the U or V substrate drop.

A primary aim of this study was to reveal how guinea fowl integrate the mechanics and control of running by investigating the immediate response of the system to an unexpected perturbation to steady forward locomotion. Consequently, we designed the experiment to create a rapid perturbation to steady forward running that was large enough to alter COM dynamics, yet as simple and unexpected as possible. To achieve this, we designed the runway to appear completely level to the bird, so that it anticipated maintaining a steady run. The birds usually took 1 step on the lower height before returning to the original substrate height; however, because the force plate was approximately equal to a step length for these birds, they sometimes (4 of 19 trials) took 2 steps on the lower height, depending on their step placement as they encountered the drop perturbation. However, we analyzed only the perturbed (first) step. We do not analyze or interpret subsequent steps except to state whether or not the birds fell down, because the behavior of the animal beyond the first step depends on a multitude of factors that could not be controlled in the context of this experiment.

Previous 'false floor' perturbation studies in humans and monkeys have demonstrated through electromyographic (EMG) measurements that this type of protocol can successfully 'fool' subjects and elicit an unanticipated response (Dyhre-Poulsen and Laursen, 1984; McDonagh and Duncan, 2002). In the current study, the ΔH of 8.5 cm was $41\pm1\%$ of the normal mid-stance limb length for the birds. The tissue paper broke at a relatively low force of 6 N, which is approximately 30% of the bird's body weight BW. Although we could not measure the force between the foot and the tissue paper during the experiment, we estimated the associated impulse to be 0.06 Ns, based on the paper breaking force, the time for the foot to break through (16±4 ms, observed from the video recordings), and assuming a half sine wave for the time-course of force development. This is 2% of the GRF impulse of steady running, and likely to have had a negligible direct effect on the motion of the COM, although it could have triggered a reflex response. To check for a learning effect in U trials, we compared sequential U trials using repeated-measures ANOVA on kinematic variables. Initial leg length, COM velocity and change in COM height in the perturbed step did not significantly differ among sequential hidden drop trials (P=0.80, 0.14 and 0.58, respectively). Our results showed no evidence of a behavioral change over sequential hidden drop trials, whereas the behavior of the animals differed markedly when they were allowed to see the upcoming ΔH (V trials). Consequently, we conclude that the hidden drop trials were unexpected and the birds did not learn to anticipate U perturbations over the course of the experiment.

The purpose of the V trials was to provide a general comparison to the hidden drop situation, in hope that it will yield insight into the effect of removing visual feedback. The behavior of the animals was less stereotyped during V trials,

and they often came to a complete stop while negotiating the change in substrate height. Consequently, although we made general observations on the behavior (whether the bird stumbled, fell or came to a stop) for all V trials, we reserved a detailed analysis of V trials to those in which the bird moved continuously across the runway (10 of 20 total trials recorded).

Data collection and measurements

Ground reaction forces (GRF), measured in the vertical (f_v) and fore–aft (f_h) directions, were recorded at 5000 Hz and synchronized to high-speed digital video (Redlake Motionscope PCI 500, Cheshire, CT, USA) recorded in both lateral views at 250 Hz.

Points located at the middle toe, tarsometatarsophalangeal joint (TMP), ankle, knee, hip, synsacrum, and the approximate body COM were digitized using custom software written in Matlab (release 13, Mathworks Inc., Natick, MA, USA). These coordinate data were smoothed and interpolated to 5000 Hz using predicted mean square error (MSE) quintic spline (Walker, 1998; Woltring, 1985, 1986). The digitized position of the COM was used to reduce the error in initial velocity values required for calculation of COM mechanics from the force platform measurements, as described in detail below.

Force plate data were low pass filtered using a zero-phase fourth-order digital Butterworth filter with a cut-off frequency between 90–100 Hz. The vertical (j_v) and fore–aft (j_h) components of the GRF impulse were calculated by numerical integration of the GRF components over the period of ground contact (t_c). The magnitude ($|\mathbf{J}|$) and angle (ϕ, measured relative to horizontal) of the resultant impulse vector ($\mathbf{J}$) were determined by:

$$|\mathbf{J}| = (j_h^2 + j_v^2)^{1/2} \tag{1}$$

and

$$\phi = \tan^{-1}(j_v/j_h) . \tag{2}$$

Calculation of COM mechanics

During steady forward locomotion, the vertical energy ($E_V = E_P + E_{Kv}$) and horizontal energy ($E_H = E_{Kh}$) of the COM are each conserved over the course of a step. During the aerial phase of steady running, the COM reaches an apex where E_{Kv} is zero and E_P is at a maximum. In this study, we measured the mechanical energy of the body, beginning from the COM apex prior to the perturbation to peak aerial phase COM height following the perturbation. This definition allows straightforward characterization of the extent to which COM mechanics have deviated from stable, steady locomotion. If the GRF impulse during the perturbed stance is insufficient to support body weight, the body continues to fall downward at end of stance. In this case there is a net gain in E_{Kv}, no subsequent apex occurs, and the aerial phase peak in COM height occurs at the beginning of the aerial phase. The ΔE_{Kv} must be absorbed or converted to another form during the next stance phase to return the body to stable locomotion, because total vertical energy ($E_V = E_P + E_{Kv}$), total horizontal energy ($E_H = E_{Kh}$) and total mechanical energy (E_{com}) can change only during the period of ground contact.

Vertical and fore–aft instantaneous accelerations (a_v, a_h, respectively) were obtained from the measured GRF and body mass (M_b):

$$a_h = f_h/M_b ,$$ (3)

$$a_v = g + f_v/M_b ,$$ (4)

where g is the vertical acceleration due to gravity. These expressions can be integrated once with respect to time to provide instantaneous velocities (v):

$$v_h = V_{i,h} + a_h dt ,$$ (5)

$$v_v = V_{i,v} + a_v dt ,$$ (6)

with initial velocity conditions ($V_{i,h}$, $V_{i,v}$) as integration constants. Eqn 5 and 6 can be integrated again to provide instantaneous positions (s):

$$s_h = S_{i,h} + v_h dt ,$$ (7)

$$s_v = S_{i,v} + v_v dt ,$$ (8)

given initial positions for integration constants ($S_{i,h}$, $S_{i,v}$). Instantaneous kinetic energy (E_{Ktot}) and gravitational potential energy (E_P) can thus be derived from Eqn 5, 6 and 8 (Cavagna, 1975):

$$E_K = 1/2 M_b(v_h^2 + v_v^2) ,$$ (9)

$$E_P = M_b g s_v .$$ (10)

Initial velocity conditions

The initial velocity conditions ($V_{i,h}$ and $V_{i,v}$) required are critical: a small error in V_i results in a progressive error of position over time. Because of this, calculated whole-body energies are highly sensitive to V_i. These initial velocity conditions must be derived from kinematic data obtained from high-speed video, photocells or some other means independent of the force platform. Since movement of appendages and viscera shift the COM location, tracking the COM position precisely from a constant morphological position is problematic. For steady locomotion, average velocities are close enough to V_i values that they may be used as V_i values without causing substantial error (e.g. Cavagna, 1975; Cavagna et al., 1977; Heglund et al., 1982). Consequently, traditional methods assume $V_{i,v}$ is equal to zero and $V_{i,h}$ is equal to average horizontal velocity. However, since we are particularly interested in the stride-to-stride variation in COM energy oscillations during unsteady locomotion, the method used to obtain V_i for studies of steady locomotion is insufficient. Previous methods to limit the effect of V_i error include (1) smoothing the position kinematics prior to differentiation, or (2) taking an average initial velocity from several frames (e.g. Roberts and Scales, 2002). However, the number of frames to be included, or the degree of smoothing required, is not readily apparent. In addition, simple averaging fails to take into account the acceleration due to gravity prior to contact with the force platform.

To best utilize the kinematic data available, we developed a path-matching technique to minimize the error in V_i. Without *a priori* knowledge of systematic error in kinematics, the 'kinematic' COM position for each field is presumed equally prone to digitizing error. In the path matching technique, the $V_{i,h}$ and $V_{i,v}$ are each iteratively adjusted to minimize the sum
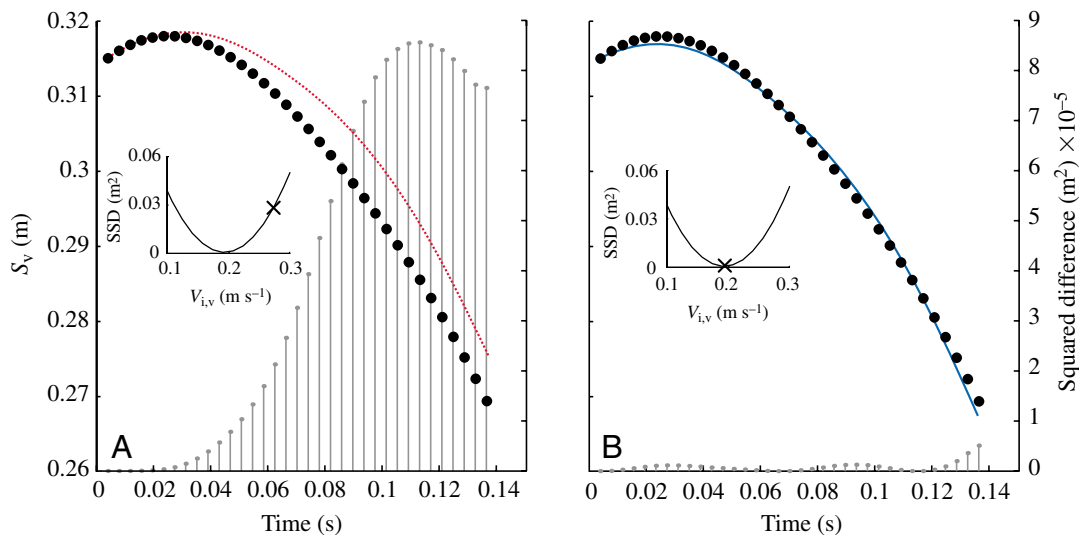

Fig. 2. Correction of initial velocity conditions for calculation of the guinea fowl's COM path. Initial velocity conditions derived using standard methods result in a path that diverges considerably from kinematic observation. The calculated vertical position (A, broken red line) and the observed kinematic estimates (black dots) relate to the left axes in A and B, and show a discrepancy represented as the squared difference at each point (grey vertical bars, right axes in A and B). The sum of the squared differences through the step period (SSD, inset in A and B) is used as a statistic for selecting the $V_{i,v}$ that minimizes the divergence between the two vertical paths. The V_i values resulting in the closest path match for the vertical (B, solid blue line) and horizontal components (not displayed, but identical principles), are then used as the integration constants in the calculation of instantaneous velocities from the ground reaction force data (Eqn 5, 6).

Fig. 3. (A) COM paths over a step cycle for one individual during the three treatments: control (C), unexpected substrate drops (U) and visible substrate drops (V). All U trials for this individual are shown to illustrate within-subject variation (solid blue lines), with a representative V trial (dotted red line) and C step (broken green line) drawn for comparison. Thin gray lines represent the aerial phase of the step. In (B) the periods of analysis are schematically illustrated. In all cases, the COM trajectory was calculated between subsequent aerial phase peaks in COM height. During level running this corresponded to subsequent COM apexes, where E_{Kv} is zero and E_P is at a maximum. However, in the U and V

trials the COM generally did not return to stable periodic motion within the perturbed step, and the COM was often moving downward at the beginning of the aerial phase following the perturbation. In these cases, there was a net gain in E_{Kv}, and the aerial phase peak in COM height occurred at the beginning of the flight phase. This ΔE_{Kv} must be dealt with during the next stance phase because total vertical energy ($E_V = E_P + E_{Kv}$), total horizontal energy ($E_H = E_{Kh}$) and total mechanical energy (E_{com}) can change only during ground contact.
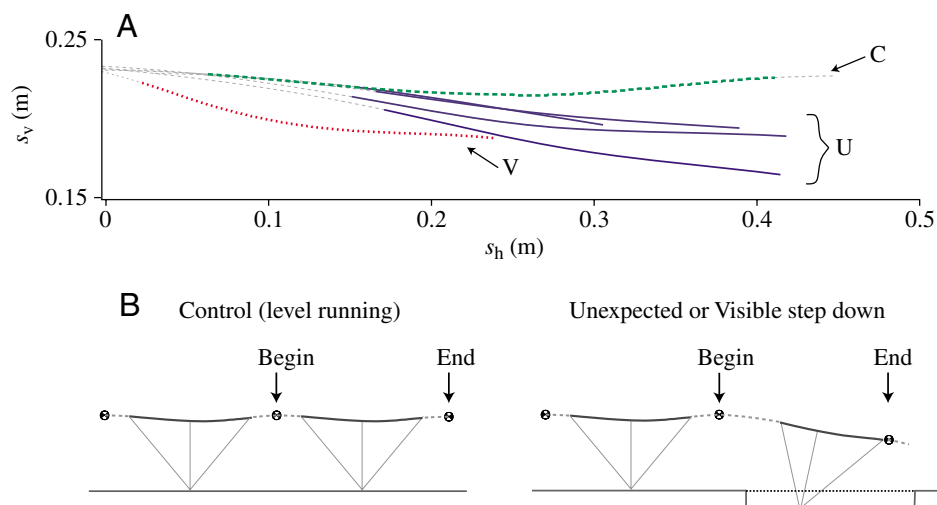
of the squared differences (SSD) between the paths observed from kinematics and those derived using force platform data. This method allows the small discrepancies between kinematic and force plate derived COM trajectories that are expected due to motion of limbs and viscera, but prevents progressive divergence of the two paths, as illustrated in Fig. 2. For a steady running trial, this method of path-matching to correct V_i results in COM trajectories that are nearly identical to those calculated using the traditional technique (described above). In the control trial illustrated in Fig. 3, the difference in V_i values between the two techniques was 0.05 m s^{-1} and –0.06 m s^{-1} for $V_{i,h}$ and $V_{i,v}$, respectively. This resulted in a difference in the final force plate derived positions of 0.5 cm and 1.0 cm in horizontal and vertical directions. For the unexpected and visible step-down trials (U and V), the difference between V_i values resulting from the path-matching technique and those derived from kinematics alone (using the method of Roberts and Scales, 2002) averaged 0.18±0.02 m s^{-1} and 0.19±0.02 m s^{-1} for $V_{i,h}$ and $V_{i,v}$, respectively. The final force plate derived positions calculated, based on unadjusted kinematic V_i values, diverged by 6.07±1.27 cm and 4.19±0.65 cm from the observed fore–aft and vertical kinematic positions, whereas those calculated using the path-match corrected V_i values diverged by an average of 0.48±0.06 cm and 0.50±0.05 cm, respectively.

Characterization of perturbation response patterns

During steady forward locomotion, the primary requirement of the limb is to provide the GRF impulse magnitude (|**J**|, the summed GRF over the stance period) necessary to reverse the increase in vertical momentum due to gravity, and thus support body weight. If the limb is unable to produce the necessary impulse, the body falls and a net conversion of E_P to E_{Kv} occurs, although total E_V is unchanged. The GRF impulse is

directed vertically (net horizontal impulse equals zero) unless actuation of the limb is required, as in acceleration or deceleration (e.g. Lee et al., 1999; Roberts and Scales, 2002). We measured impulse direction (ϕ) relative to horizontal; a value of 90° indicates a vertically directed **J**. In summary, a reduced |**J**| indicates a decrease in body weight support and a net conversion of E_P to E_{Kv} over the course of a step, whereas an altered ϕ (deviation from 90°) indicates altered E_{Kh}, either through conversion of E_V to E_{Kh} or actuation by muscles of the limb.

The perturbation led to a net loss in E_P and total E_V ($E_P + E_{Kv}$) during the perturbed step in all U trials. We term this ΔE_V the 'perturbation energy'. We observed considerable variability in the COM energy patterns associated with this ΔE_V. Consequently, we investigated the link between |**J**|, ϕ, and the COM dynamics based on the expected relationships between **J** and the mechanics of support (above). The trials were separated into three categories, based on whether most (>50%) of the ΔE_P was converted to E_{Kh}, to E_{Kv}, or absorbed through muscular work ($-\Delta E_{com}$). A cluster analysis was used to test whether the variables |**J**| and ϕ significantly distinguished the trials in these three energy response categories. Additionally, we looked at how |**J**| and ϕ related to two energy ratios that characterize how well the limb supported body weight and how consistent limb function was with a spring. The vertical energy ratio ($\Delta E_{Kv}:\Delta E_P$) is a measure of how well the limb supported body weight. A value of zero indicates full support of body weight, whereas a value of 1.0 indicates freefall with no support of body weight. The perturbation energy ratio ($\Delta E_{com}:\Delta E_V$) characterizes the extent of energy redistribution vs actuation by the limb. A value of zero indicates that the perturbation energy was converted to horizontal kinetic energy ($\Delta E_V \rightarrow \Delta E_{Kh}$), with no net muscular work. This is consistent with spring-like limb function. In contrast, values approaching

Individual	Mass (kg)	Σl_{seg} (m)	HH (m)	Trials		
				C	U	V
1	1.10	0.31	0.19	2	5	2
2	1.52	0.32	0.20	2	4	2
3	2.06	0.33	0.20	2	2	3
4	2.41	0.37	0.22	2	3	2
5	2.64	0.37	0.22	2	5	1

Σl_{seg}, sum of limb segment lengths; HH, standing hip height; C, U and V trials, control, unexpected drop and visible drop trials, respectively.

1.0 indicate increasing absorption of the perturbation energy through negative muscular work ($\Delta E_V \rightarrow \Delta E_{com}$). A value of 1.0 indicates that all of the perturbation energy has been absorbed by the limb. A value greater than 1.0 indicates additional energy loss and deceleration ($-\Delta E_{Kh}$), and a negative value indicates energy production and acceleration ($+\Delta E_{Kh}$).

Statistical analysis

For statistical analysis all mechanical variables were made dimensionless by normalizing to body mass (Table 1), the acceleration of gravity (**g**) and total leg length (Table 1, Σl_{seg}, where l_{seg} is length of limb segment; McMahon and Cheng, 1990). A two-way mixed model ANOVA was used to assess the effect of treatment (C, U, V) and individual on net change in total mechanical energy (ΔE_{com}), gravitational potential energy (ΔE_P), fore–aft kinetic energy (ΔE_{Kh}) and vertical kinetic energy (ΔE_{Kv}), as well as initial velocity ($V_{i,h}$), ground contact time (t_c), impulse magnitude ($|\mathbf{J}|$), and impulse direction (ϕ). To characterize the mechanical differences between different 'energy exchange modes' during the U trials, a one-way ANOVA was used with 'energy exchange mode' as the factor and ΔE_{com}, ΔE_P, ΔE_{Kh}, ΔE_{Kv}, $V_{i,h}$, t_c, $|\mathbf{J}|$, ϕ as dependent variables. To account for the number of simultaneous ANOVAs performed, the P-values for each test were adjusted using the sequential Bonferroni technique or the Tukey Honestly Significant Difference *post hoc* test (THSD). An adjusted P-value ≤ 0.05 was considered statistically significant. Repeated-measures ANOVA was used to test for a learning trend in Δs_v, $V_{i,h}$ and initial limb length during consecutive U trials. Relationships between individual pairs of variables were evaluated using least-squares linear regression or Student's t-test, where appropriate. All tests were performed using Systat (version 10.2 for the PC). Average values given in the text are means ± s.e.m.

Results

Behavior and center of mass trajectory during hidden and visible substrate drops

Guinea fowl were unable to compensate fully for the ΔH within the perturbed step during unexpected substrate drops,

but were successful in maintaining overall dynamic stability. In none of the trials did the COM trajectory resemble steady, linear spring-mass operation during a U perturbed step. That is, there was a significant ΔE_P in all U trials and a corresponding increase in kinetic energy (ΔE_{Ktot}) or absorption of energy ($-\Delta E_{com}$). Although the birds did not completely accommodate to an unexpected ΔH of this magnitude without a deviation from the steady COM trajectory, they were quite successful in maintaining dynamic stability, as the birds did not fall or come to a stop in any U trial. A stumble (without falling) occurred only once in the 19 U trials during the step up following the perturbation. Although we cannot make any conclusions about what the birds perceived during the perturbation, they did not typically slow down or change their behavior dramatically when they stepped back up to the original height (see Movies 2–4 in supplementary material). When there was a second step in the drop region during U perturbations (4 of 19 trials), the birds typically placed the foot for contact at the height of tissue paper, not at the force plate height, as though they had not altered their step placement substantially from the original trajectory. Furthermore, the change in average forward speed on the original runway height following the perturbation was only $0.1\,\mathrm{m\,s^{-1}}$, and not significantly different from the change in forward speed during C trials before and after the force plate (two-tailed t-test, $P=0.63$). Birds did not exhibit any trends in Δs_v or initial limb length during the perturbed step over the course of sequential U trials ($P>0.05$, see Materials and methods), indicating that the tissue paper-camouflaged perturbations remained unexpected.

The COM trajectory during an unexpected perturbation was variable, ranging from an initial falling phase followed by leveling off, to falling in a nearly ballistic path for the entire step (Fig. 3). At the end of the perturbed step, the COM had fallen to a lower height and velocity of the COM had increased (Fig. 4B). This fall in s_v resulted in a ΔE_P that averaged $-1.0\pm0.2\,\mathrm{J}$ (Fig. 5), did not significantly differ across individuals (Table 2, $P=0.425$), and corresponded to a net

Table 2. *ANOVA for effects of treatment and individual*

Variable	Treatment			Individual		
	d.f.	F	P	d.f.	F	P
ΔE_P	2	32.06	<0.001*	4	1.01	0.425[ns]
ΔE_{Kh}	2	6.18	0.024*	4	0.97	0.447[ns]
ΔE_{Kv}	2	21.46	0.001*	4	1.35	0.288[ns]
ΔE_{com}	2	1.95	0.204[ns]	4	0.72	0.586[ns]
Speed	2	9.95	0.007*	4	2.19	0.102[ns]
t_c	2	18.85	0.001*	4	6.73	0.001*
$|\mathbf{J}|$	2	35.24	<0.001*	4	1.18	0.346[ns]
ϕ	2	5.21	0.036[ns]	4	0.57	0.691[ns]

*Significant difference after Bonferroni correction.

For an explanation of symbols, see text and List of symbols and abbreviations.

change in COM height (Δs_v) averaging -5.1 ± 0.3 cm, or 60% of ΔH (Fig. 6). For the unexpected perturbation trials, most of the ΔE_P (94%) occurred during the stance phase of the perturbed step, when the limb was in contact with the force platform, not during the time between tissue break-through and ground contact (Fig. 7), which averaged 26 ± 1 ms. This ΔE_P did not result solely from inadequate weight support during the perturbed step (leading to conversion of E_P to E_{Kv}); in all cases there was a net loss in total vertical energy ($E_V=E_P+E_{Kv}$), associated with a combination of net energy absorption by the limb and conversion of E_V to E_{Kh} (Fig. 7). The ΔE_P during the perturbed step represents a large change in energy compared

to the oscillations associated with steady running (Control $\Delta s_{v,max}=-0.4\pm0.5$ cm, $\Delta E_{P,max}=-0.08\pm0.1$ J).

Similar to the U perturbation trials, in visible (V) substrate drops, guinea fowl were usually unable to compensate completely for the ΔH within the perturbed step (preventing a significant ΔE_P or ΔE_{com}), although they did successfully do so in two of the 20 recorded V trials. In general, however, the behavior during V trials differed markedly from and was less stereotyped than the behavior during U trials. In six of 20 recorded V trials the bird came to a complete stop while negotiating the step, and in another four the bird stumbled (falling twice and re-stepping twice) during the step back up to the original runway height. Among the 10 trials in which the bird moved continuously across the runway, the ΔE_P and ΔE_{com} were within the 95% confidence interval for the control means for two trials. Therefore, it is possible for the birds to accommodate a perturbation of this magnitude to maintain a steady spring-like trajectory at the original COM height in some instances when they could see the upcoming change. However, on average they were not significantly more successful in preventing a ΔE_P than during U trials. When the bird moved continuously over the drop section, COM trajectories fell during the first half of stance and subsequently leveled off (Fig. 3). The ΔE_P tended to be less than during U drops, but not significantly so (THSD, $P=0.232$). The Δs_v averaged -4.2 ± 1.2 cm, or 49% of ΔH, representing an average ΔE_P of -0.8 ± 0.3 J (Fig. 5). Similar to U substrate drops, most (82%) of the ΔE_P occurred during limb support rather than during the flight phase approaching the lower substrate height. However, in V trials the net loss in E_P and E_V was associated with net energy absorption by the limb (Fig. 5), so, unlike U substrate drops, the velocity was not greater at the end of the perturbed step (Fig. 4).
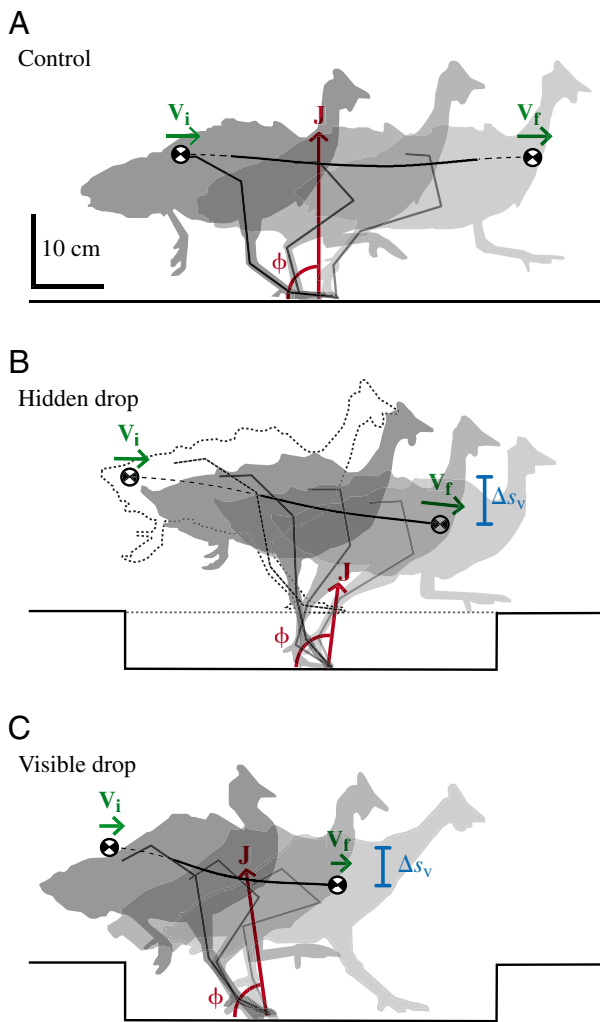
Fig. 4. Summary of COM mechanics during C, U and V treatments (A,B,C, respectively). Silhouettes of the bird with corresponding limb stick figures at three points during the perturbed step: toe-down, mid-stance, and toe-off. The broken silhouette and stick figure represent the time of tissue paper contact in the U treatment. The COM path is overlaid for the time interval illustrated in Fig. 3B, along with the corresponding net change in height (Δs_v, blue), GRF impulse vector (**J**, red, summed over the stance phase), and initial and final velocity vectors (**V$_i$** and **V$_f$**, respectively, green) to illustrate differences among treatments.
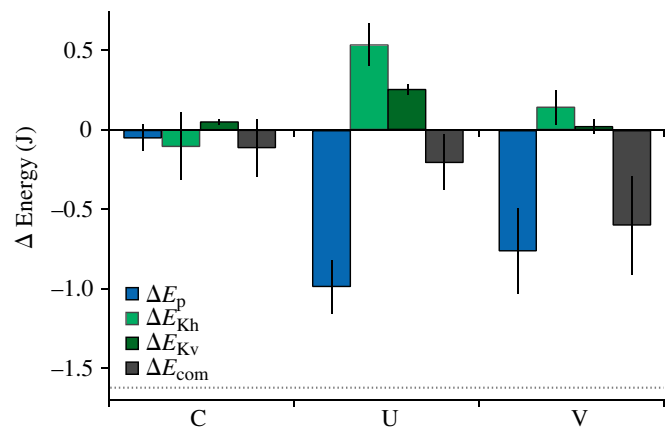
Fig. 5. Net changes in gravitational potential (ΔE_P), horizontal and vertical kinetic (ΔE_{Kh} and ΔE_{Kv}, respectively), and total mechanical energy (ΔE_{com}) of the COM over the course of one step for the C, U and V treatments. The broken gray line indicates the ΔE_P that would occur if the birds fell the entire substrate drop ($\Delta H=8.5$ cm). Values are means ± s.e.m for all individuals ($N=5$). The net changes in energy were determined over the time interval illustrated in Fig. 3B.
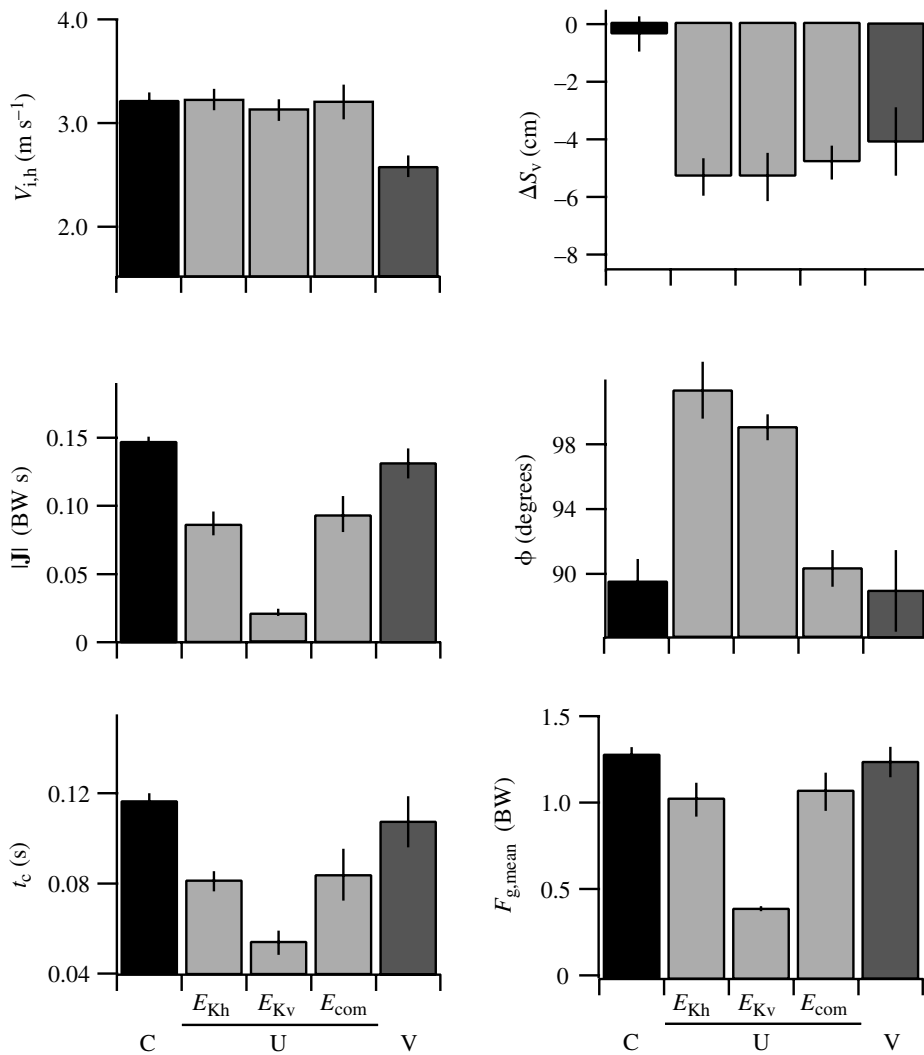
Fig. 6. Comparison of mechanical variables across C (black bars), U (pale grey bars) and V (dark grey bars) treatments, with U trials subdivided into energy exchange modes. Values are means ± s.e.m for all instances of each response. Initial horizontal velocity ($V_{i,h}$) was measured at the COM apex during the flight phase prior to the measured step ('begin' point in Fig. 3B). The net change in COM height (Δs_v) was determined over the time interval illustrated in Fig. 3B. The other mechanical variables were measured over the period of ground contact.

Energy exchange modes during unexpected step perturbations

On average, most of the ΔE_P was converted to a net change E_{Ktot} during unexpectedly perturbed steps, with the majority of the ΔE_{Ktot} occurring as ΔE_{Kh}, accelerating the animal forward (ΔE_{Kh}; Fig. 5). However, as noted above, the COM paths during U perturbations were quite variable (Fig. 3). The energy exchange patterns associated with these different COM trajectories can be separated into three general categories based on whether most (>50%) of the ΔE_P was converted to E_{Kh}, E_{Kv} or absorbed through negative muscle work ($-\Delta E_{com}$). Consistent with the expected relationship between $\mathbf{J}$ and the mechanics of support (see Materials and methods), we found that the variables |$\mathbf{J}$| and ϕ are sufficient to significantly distinguish the three energy response categories in a cluster analysis ($P<0.001$). In 'E_{Kh} mode', |$\mathbf{J}$| is lower than during level running and directed forward ($\phi>90°$; Fig. 6). Most of the ΔE_P is converted to E_{Kh}, with a small increase in E_{Kv} as well as a small net production of energy (Fig. 7B). In 'E_{com} mode', |$\mathbf{J}$| is similar to 'E_{Kh} mode', but directed near vertical or rearward ($\phi \leqslant 90°$; Fig. 6), and most of the ΔE_P is absorbed through negative work $-\Delta E_{com}$ (Fig. 7C). Finally, in 'E_{Kv}

mode', |$\mathbf{J}$| is very low (Fig. 6) and the ΔE_P is simply converted to E_{Kv} as the bird's COM falls (Fig. 7D). Out of 19 total unexpected drop trials, over half the ΔE_P was converted to E_{Kh} in 9 trials (47%), absorbed as $-\Delta E_{com}$ in 7 trials (37%), and converted to E_{Kv} in 3 trials (16%). All five individuals exhibited 'E_{Kh} mode', four exhibited 'ΔE_{com} mode', whereas only one exhibited 'E_{Kv} mode' (Table 3).

COM mechanics during hidden vs visible perturbations

As suggested by the COM trajectories, different mechanisms were used to negotiate the ΔH in unexpected and visible substrate drops. Whereas in U trials the magnitude of the GRF impulse (|$\mathbf{J}$|) was significantly lower than in level running (Fig. 6; THSD, $P<0.001$), |$\mathbf{J}$| in V trials was greater than U trials and similar to level running (Fig. 6; THSD, $P=0.002$). Reduction in both t_c and mean force during contact ($F_{g,mean}$=|$\mathbf{J}$|/t_c, Fig. 6) contributed to this reduction in weight support in U trials, leading to an (downward) increase in E_{Kv} (Fig. 5, $P<0.001$). The greater weight support in V trials resulted in a smaller ΔE_{Kv} (Fig. 5; THSD, $P=0.021$). In 'E_{Kh} mode' and 'E_{Kv} mode' U trials, $\mathbf{J}$ was also directed forward,
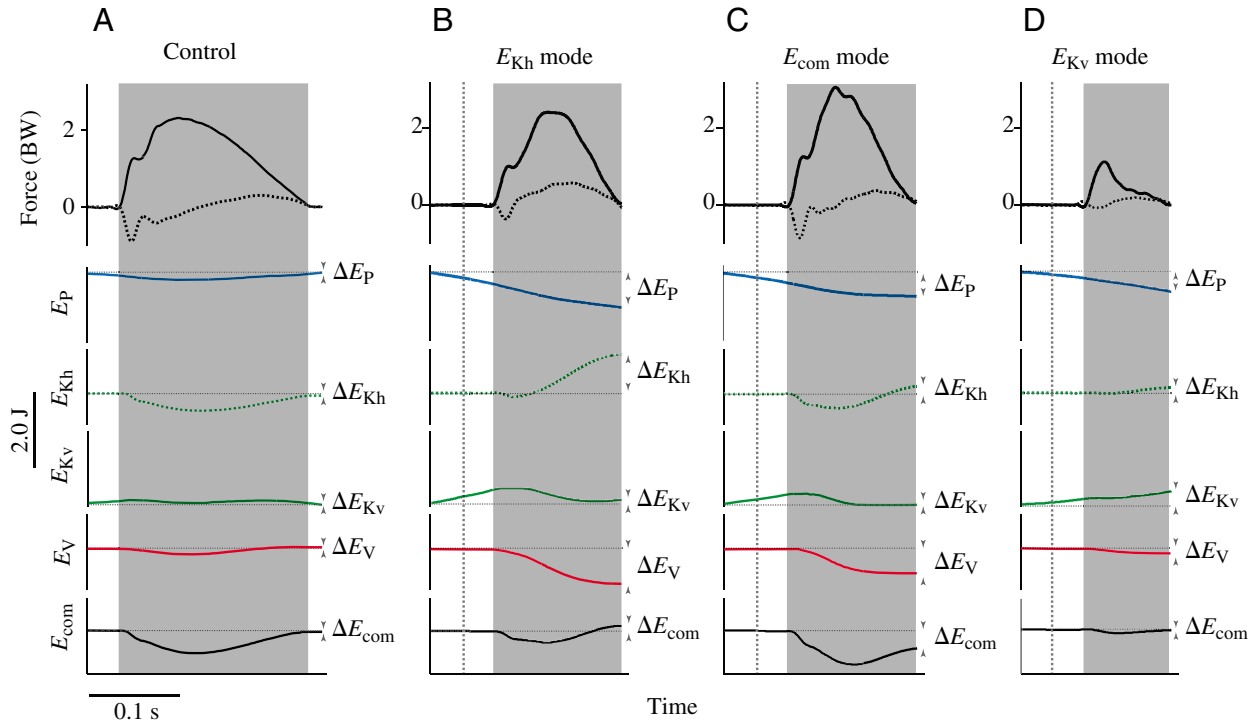
Fig. 7. Ground reaction force (top), and COM energy changes (below) over time for the step period for representative trials, comparing steady, spring-like dynamics of level running (A) to the three energy responses observed during U perturbations: (B) 'E_{Kh} mode', (C) 'E_{com} mode' and (D) 'E_{Kv} mode'. The time interval shown is that illustrated in Fig. 3B. Bold and dotted lines in top panels indicate instantaneous vertical (f_v) and horizontal (f_h) GRF, respectively. The dotted vertical lines indicate time of tissue paper contact, and gray bars indicate duration of ground contact. Gravitational potential (E_P), horizontal kinetic (E_{Kh}) and vertical kinetic (E_{Kv}) energies are shown over time, with dotted horizontal lines to indicate the initial energy. Two energy sums, total vertical energy ($E_V=E_P+E_{Kv}$) and total COM energy ($E_{com}=E_P+E_{Ktot}$), allow distinction between energy conversion (e.g. $E_P \rightarrow E_{Kh}$ as in B) and net energy absorption (as in C). Note that at the point of ground contact in U trials (B–D), E_{Kv} is greater than in level running (A) because the body falls during the perturbation.

so that ϕ was larger than during level running (Fig. 6; THSD, $P=0.045$). In contrast, although ϕ was variable in V trials, it tended to be directed vertically or rearward, such that ϕ was not significantly different from control (Fig. 6; THSD, $P=0.895$). Likewise, during V trials, most of the ΔE_P was absorbed by the limb and body in the form of negative ΔE_{com} (Fig. 5), similar to 'ΔE_{com} mode' U trials.

Body weight support and limb actuation distinguish energy exchange modes

Although these 'energy mode' categories are conceptually useful, the COM mechanics actually occur across a continuum that can be illustrated by examining the relationship between the magnitude ($|\mathbf{J}|$) and direction (ϕ) of the GRF impulse and two energy ratios: (1) the vertical energy ratio ($\Delta E_{Kv}:\Delta E_P$) and

Table 3. *ANOVA test for effect of energy exchange mode*

Variable	Mode			Individual	Mode frequency		
	d.f.	F	P		E_{Kh}	E_{Kv}	E_{com}
ΔE_P	2	0.42	0.667[ns]	1	1	3	1
ΔE_{Kh}	2	18.09	<0.001*	2	2	0	2
ΔE_{Kv}	2	9.95	0.002*	3	2	0	0
ΔE_{com}	2	12.33	0.001*	4	2	0	1
Speed	2	0.05	0.956[ns]	5	2	0	3
t_c	2	2.54	0.11[ns]				
$|\mathbf{J}|$	2	7.09	0.006*				
ϕ	2	10.65	0.001*				
Δs_v	2	0.21	0.816[ns]				

Only unexpected drop trials are included.
For an explanation of symbols, see text and List of symbols and abbreviations.

(2) the perturbation energy ratio (ΔE_{com}:ΔE_V). The vertical energy ratio is a measure of how well the limb supported body weight. A value of zero indicates full support of body weight, whereas a value of 1.0 indicates freefall. The perturbation energy ratio characterizes the extent of energy redistribution *vs* actuation by the limb. A value of zero indicates that the perturbation energy was converted to horizontal kinetic energy ($\Delta E_V \rightarrow \Delta E_{Kh}$), with no net muscular work. This is consistent with spring-like limb function. In contrast, values approaching 1.0 indicate increasing absorption of the perturbation energy through negative muscular work ($\Delta E_V \rightarrow \Delta E_{com}$). The vertical energy ratio was significantly correlated with impulse
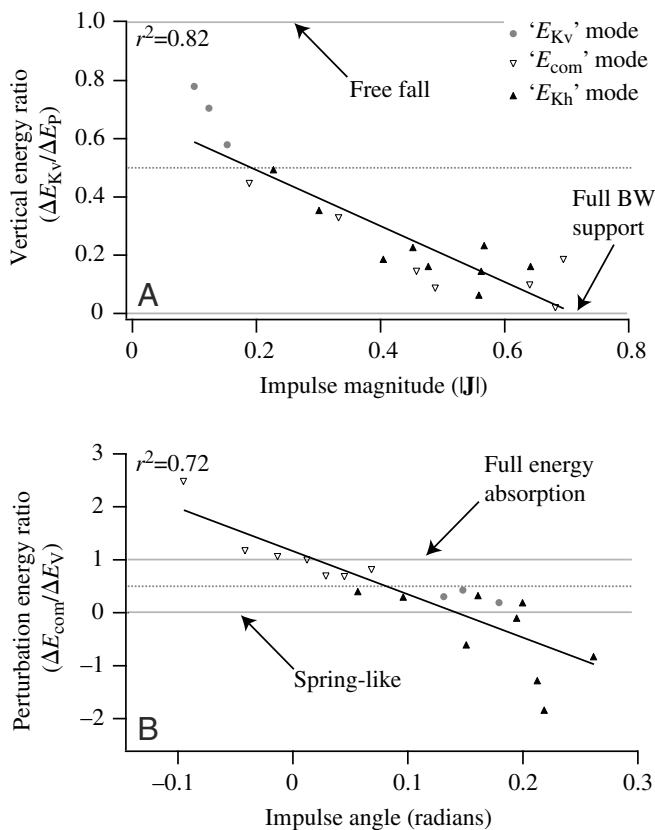

Fig. 8. The COM mechanics occur along a continuum related to how well the limb supported body weight BW and whether it produced or absorbed net energy. Different symbols represent different 'energy mode' categories. The GRF impulse magnitude and direction (|**J**| and ϕ) distinguish the different response patterns, as illustrated by their relationship with two energy ratios. (A) The vertical energy ratio (ΔE_{Kv}:ΔE_P) strongly correlates with |**J**|, and indicates the level of BW support (0 indicates full BW support, 1.0 indicates free fall). We categorized trials in which the E_V ratio ≥0.5 as 'E_{Kv}' mode (dotted line). (B) The perturbation energy ratio (ΔE_{com}:ΔE_V) correlates with ϕ, and distinguishes energy redistribution *vs* actuation by the limb. A value of zero indicates that the ΔE_V was converted to E_{Kh} ($\Delta E_V \rightarrow \Delta E_{Kh}$), with no net muscular work, consistent with spring-like limb function. A value of 1.0 indicates that all of ΔE_V is absorbed, a value >1.0 indicates deceleration ($-\Delta E_{Kh}$) and a value <0 indicates acceleration ($+\Delta E_{Kh}$). We categorized trials in which the perturbation energy ratio ≥0.5 as 'E_{com}' mode (dotted line).

magnitude, |**J**| (Fig. 8, r^2=0.82), and distinguished 'E_{Kv}' trials from 'E_{Kh}' and 'E_{com}' trials, whereas the perturbation energy ratio was significantly correlated with impulse direction, ϕ (Fig. 8; r^2= 0.72), and distinguished energy absorbing (ΔE_{com}) trials from 'E_{Kh}' and 'E_{Kv}' trials. Altered contact time, t_c, and $F_{g,mean}$ both contributed to the variation in |**J**| in U trials (Fig. 6). In summary, each energy mode is characterized by a distinct combination of altered GRF impulse direction and magnitude during stance; however, these two variables actually describe distinct aspects of the COM dynamics along a continuum.

Possible explanations for the observed variation in response dynamics include variation in the body mass, leg length or initial forward speed of the bird. Differing initial conditions could also cause the body to respond differently to a perturbation of the same magnitude. Yet, we found that neither $V_{i,h}$ nor size sufficiently explains the occurrence of different energy exchange modes during U trials. None of the modes differed significantly in terms of the animal's initial horizontal velocity, $V_{i,h}$ (Fig. 6, Table 3). Further, the average perturbation energy ratio (which distinguishes 'E_{com}' from 'E_K' modes) did not significantly differ across individuals when grouped by limb length (one-way ANOVA, THSD, P=0.586). However, the smallest individual (Table 1, individual 1) had a significantly higher vertical energy ratio on average during U trials than the other individuals (one-way ANOVA, THSD, P=0.005). Therefore, the smallest bird did exhibit a response pattern different from the other animals; however, size did not influence the extent of energy absorption or conversion to E_{Kh} in the U perturbation trials.

Discussion

How do guinea fowl respond to an unexpected change in substrate height?

At present, we know little about the control strategies that animals use to maintain stability in the face of the types of perturbations they face in their natural environment. Consequently, using a relatively simple approach, we examined the mechanical energy changes of a running guinea fowl's body upon experiencing a sudden drop in terrain height to study control mechanisms that animals may use to stabilize themselves. We compare the response following a camouflaged, unexpected ΔH to that in which the ΔH is visible. The extent to which guinea fowl maintain body weight support and spring-like limb function provides insight into the mechanisms used by these animals to achieve robust dynamic stability. To avoid instability leading to a fall upon encountering a sudden ΔH, the bird must dissipate energy, convert it to another form, or perform both in combination. As outlined in the Introduction, it is useful to consider three hypothetical responses to the perturbation that represent mechanical extremes: (1) complete compensation and maintenance of a steady spring-like trajectory with no net changes in component energies (E_P, E_{Kh}, E_{Kv}) or total mechanical energy (E_{com}) (2) a ΔE_P converted to ΔE_{Ktot},
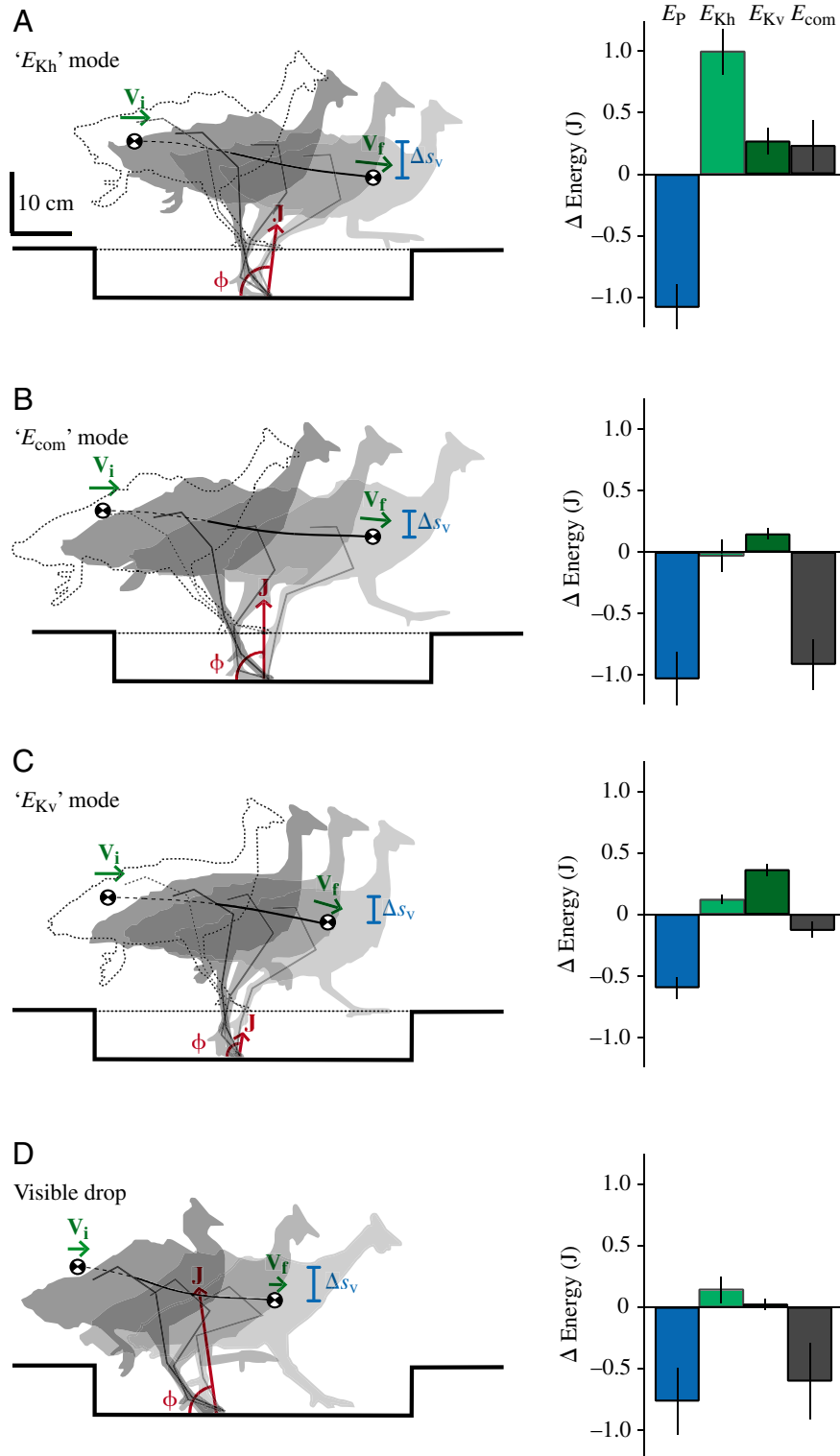
Fig. 9. Comparison of COM mechanics among different U perturbation responses (A–C). (D) The V perturbation response. On the left, silhouettes of the bird with corresponding limb stick figures are shown at three points: toe-down, mid-stance and toe-off. Broken silhouettes represent the time of tissue paper contact in U trials. The COM path is shown for the time interval illustrated in Fig. 3B, along with the corresponding net change in height (Δs_v, blue), initial and final velocity vectors ($\mathbf{V_i}$ and $\mathbf{V_f}$, respectively, green), and GRF impulse vector ($\mathbf{J}$, red, summed over the stance period). On the right, net changes in external energy shown as means ± s.e.m. for all instances of each response.

increasing the animal's velocity but still consistent with conservative mass-spring dynamics, and (3) a ΔE_P that is absorbed through negative work,, resulting in a ΔE_{com}.

We found that the response to the unexpected ΔH is a combination of the latter two possibilities (increasing E_{Ktot} and absorbing energy) with varying degrees of body weight support. These occur along a continuum that relates to the direction and magnitude of the GRF impulse exerted by the limb during the stance phase following the perturbation. Overall, the experimental evidence demonstrates that the birds are not able to fully compensate for the unexpected ΔH within the perturbed step, as they have not fully recovered to a steady COM trajectory by the end of the perturbed step, nor do they completely preserve conservative spring-mass dynamics during the response. Instead, they exhibit a combination of elastic function with net energy absorption or production by the limb.

Nevertheless, the guinea fowl are remarkably successful in maintaining dynamic stability despite the variable response to this perturbation. An 8.5 cm change in substrate height is 41% of mid-stance limb length (from hip to toe), and a stumble (a re-step on the step up following the perturbation) occurred only once in all U trials. Furthermore, the average change in velocity from the beginning to the end of the Plexiglass® runway section is not significantly greater than in C trials. Perhaps surprisingly, the birds are more likely to stumble in response to visible substrate drops. In 20 V trials, they stumbled, fell or came to a complete stop in 50% of the cases. However, they also successfully maintained a steady spring-like COM trajectory in two of the V trials (10%). This suggests that guinea fowl use different strategies for negotiating uneven terrain when they can see and anticipate the changes. Furthermore, this demonstrates that they are capable of completely adjusting limb mechanics within the perturbed step to maintain a spring-like COM trajectory in response to a ΔH of this magnitude, but only when they accurately anticipate the change based on visual information.

COM dynamics and stability during unexpected perturbations: three energy exchange modes

Rather than following symmetrical spring-like COM trajectories with no net changes in E_P, E_{Ktot} or E_{com}, as during steady running (Fig. 7A; e.g. Cavagna, 1975; Cavagna et al., 1976, 1977), guinea fowl exhibit asymmetrical COM trajectories with conversion of E_P to E_{Ktot} and absorption of E_P through negative work. The responses can be differentiated into three basic patterns during the perturbed step based on the GRF impulse magnitude and direction (|**J**| and φ, respectively). Lost E_P is (1) converted to E_{Kh} in association with a relatively large, forward directed GRF impulse, (2) absorbed by the limb muscles when the impulse magnitude is high and directed rearward, and (3) converted to E_{Kv} if the impulse magnitude is too low for substantial body weight support (Figs 8 and 9). This mechanical variation actually occurs along a continuum that can be differentiated into two relationships: (1) body weight support, related to GRF impulse magnitude, and (2) production or absorption of energy, correlated with the GRF impulse direction (Fig. 8). This variation in the dynamic response to the perturbation likely reflects variation in the limb kinetics during the response.

The three response modes are likely to affect running stability differently because they each involve different deviations from the steady COM trajectory (Fig. 9). In a general sense, an animal is successful in maintaining dynamic stability if it avoids falling and returns to steady, periodic COM motion. This requires avoiding excessive COM motions and energy oscillations. The energy absorbing response shows the largest ΔE_{com}, but the smallest ΔE_{Kv}, (Fig. 9B). In contrast, the 'E_{Kh}' mode involves a larger increase in velocity (Fig. 9A). Energy absorbed might not be recoverable, whereas additional E_K could be converted back to E_P when the bird reaches the other side of the runway 'drop' section, facilitating recovery of its original COM height. Therefore, the dumping of energy in the 'E_{com}' mode might be undesirable. Conversely, since the bird accelerates in the 'E_{Kh}' mode, it may not have time to adjust step placement or timing, increasing the risk of a catastrophic fall. Only the smallest bird exhibited the 'E_{Kv}' response, which basically constitutes a brief limb impact as the bird falls until the contralateral limb contacts the ground. In steady running E_{Kv} reaches zero when the COM reaches its peak height during the aerial phase (apex); therefore a net increase in E_{Kv} during a step means that the body has not yet returned to stable periodic bouncing motion. Thus, the larger increase in E_{Kv} associated with lower impulse magnitudes may represent a less stable response. Furthermore, a substantial increase in E_{Kv} is likely to disrupt visual and vestibular perception. Nonetheless, the 'E_{Kv}' response has the shortest contact time for the perturbed step (Fig. 6), and still allows the limb to gain proprioceptive feedback from brief ground contact, in addition to any feedback gained from the limb breaking through the tissue paper ~26 ms before contacting the force plate (and see below). This could facilitate more rapid recovery by the contralateral limb during the subsequent step.

Interdependence of mechanics and control during substrate height perturbations

Although a detailed examination of limb mechanics is planned for future studies, the current results allow some inferences about the relationship between COM and limb dynamics. In most U trials, the limb either absorbs or produces net energy during the drop in substrate height (Fig. 8B). Similarly, humans preserve spring-like motion of the COM through actuation of the limb when hopping is perturbed using a damped (viscous) surface (Moritz and Farley, 2003). These observations suggest that, in addition to elastic mechanisms, muscular work also plays an important role in the mechanical response when the limb's interaction with the environment changes dramatically.

An animal must appropriately couple limb muscle activation to the passive loading of the 'leg-spring' to run steadily forward. Observing the response to an unexpected perturbation yields insight into how animals integrate mechanics and motor control to accomplish this. According to the mass-spring model of running and hopping, loading and unloading of the 'leg-spring' is passive. However, the muscles of the limb must activate with the appropriate timing and intensity to resist ground reaction forces and provide the appropriate k_{leg}. The activation level of the limb extensors depends on a combination of feed-forward, rhythmic motor control, and proprioceptive feedback including muscle stretch (spindle organs, Ia) and muscle-tendon load (Golgi tendon organs, Ib) (reviewed by Grillner, 1975; Pearson, 2000; Pearson et al., 1998). Furthermore, whereas vertical hopping can be described by the simplest linear mass-spring model, running additionally involves retraction of the limb through an arc during stance (McMahon and Cheng, 1990; Raibert and Brown, 1984; Raibert et al., 1984). This is why the mass-spring model of running is also referred to as a 'spring-loaded inverted pendulum' (Full and Farley, 2000; Full and Koditschek, 1999). Limb retraction usually occurs through retraction of the hip (e.g. Belli et al., 2002; Gregersen et al., 1998), but is assisted by the knee in birds (Gatesy, 1999). When tuned appropriately to the loading and unloading of the 'leg-spring' during steady locomotion, limb retraction results in forward progression with a symmetrical COM trajectory (McMahon and Cheng, 1990; Raibert and Brown, 1984; Raibert et al., 1984), without the exchange of E_K and E_P associated with inverted pendulum-like action. Motor control research has demonstrated that afferent feedback from the hip flexors and ankle extensors control the duration of stance phase, maintaining limb extensor activity until the limb reaches a fully retracted position (reviewed by Grillner, 1975; Pearson et al., 1998). This simple control scheme automatically provides the appropriate coupling between muscle activation, loading of the 'leg-spring' and limb retraction during steady forward locomotion. The observed changes in COM dynamics during the unexpected perturbation likely reflect the mechanical consequences of decoupling feed-forward components from feedback and intrinsic mechanical components of this control system.

The unexpected ΔH perturbation results in a 26 ms delay in limb loading relative to that anticipated by the bird. The initial response likely reflects the interplay between the feed-forward motor pattern and the intrinsic dynamics that result from an altered relationship between the system and the environment. When humans and monkeys land on a platform after passing through a false surface, muscle activity is coordinated to the anticipated time of landing on the false surface (Dyhre-Poulsen and Laursen, 1984; McDonagh and Duncan, 2002). If feed-forward muscle activation causes the limb to retract upon tissue break-through, it will contact the ground with a more vertical posture and a smaller horizontal distance between the COM and foot (Figs 1, 9). The fraction of stance during which the COM is behind the foot is likely reduced, resulting in a diminished decelerating force on the body (Fig. 7). Consequently, the GRF impulse is directed forward relative to steady running (Figs 6, 9). Repositioning the foot relative to the COM at the beginning of stance is an effective mechanism for controlling acceleration and deceleration in bouncing gaits (Raibert and Brown, 1984; Raibert et al., 1984), and may provide intrinsic stabilization during running (Seyfarth et al., 2003). A further likely consequence of this change in geometry is reduced loading of the extensor muscle–tendon systems, resulting in decreased elastic energy storage in the limb. If the limb retracts as usual with reduced leg-spring compression compared to steady running, an inverted pendulum motion will result, leading to an exchange between E_P and E_K. Thus, one can view the E_{Kv} and E_{Kh} responses to the U perturbation as the body vaulting over the limb.

Similarly, the reduction in stance duration (t_c) in the U perturbations could result from uncoupled timing between limb retraction and limb loading. Stance phase muscle activity is maintained until the hip reaches a certain angle (reviewed by Grillner, 1975; Pearson et al., 1998). If the limb begins stance at a different angle, yet retracts at a similar rate and leaves the ground at a fixed angle, t_c will be reduced in proportion to the change in initial angle. Thus, the timing of limb retraction likely determines stance duration during ΔH perturbations. In visible substrate drops, the bird could adjust limb retraction in a feed-forward manner, restoring t_c to near control values (Fig. 6).

What leads to the reduced weight support characteristic of U perturbations? Both intrinsic mechanical and reflex feedback factors likely contribute to the decrease in $F_{g,mean}$. Intrinsic changes in musculoskeletal mechanics play an important role in stabilization: running cockroaches stabilize their COM trajectory within one step after a lateral impulsive perturbation (Jindrich and Full, 2002), and hopping humans exhibit rapid, intrinsic changes in k_{leg} when surprised by a surface of different stiffness (Moritz and Farley, 2004). In the current study, the limb is more extended and retracted at ground contact (Figs 1 and 9). The resulting increase in mechanical advantage and k_{leg} would tend to increase F_g for a given muscle force (Biewener, 1989, 2003; McMahon et al., 1987). However, the rapid joint extension and muscle shortening upon tissue breakthrough that results in the altered limb posture could also lead to reduced

muscle force through intrinsic ('preflexive') effects of the force–length and force–velocity properties of muscle (Brown and Loeb, 2000). Therefore, we hypothesize that intrinsic muscle properties contribute to reduced muscle force generation during the perturbed stance.

Nonetheless, reflexes also likely play a role; the 26 ms delay between tissue breakthrough and ground contact may be enough time for reflex action (e.g. Nichols and Houk, 1976). Reflexes play a number of roles during the support phase of locomotion: muscle stretch (spindle organs, Ia) reflexes stabilize limb trajectory, load receptor (Golgi tendon organs, Ib) reflexes influence body support, and together stretch and load generate 'reflex stiffness' (e.g. McMahon, 1984; Nichols and Houk, 1973, 1976; Pearson et al., 1998; Zehr and Stein, 1999, 2000). A likely contributor to the reduction in $F_{g,mean}$ is feedback from Golgi tendon organs upon tissue break-through and limb unloading. These proprioceptors generate positive force feedback that normally contributes to weight support (Donelan and Pearson, 2004; Gorassini et al., 1994; Hiebert et al., 1994). Consequently, inhibited muscle activity due to loss of ground support could explain the reduction in $F_{g,mean}$. Theoretical studies suggest that positive force feedback improves the stability of bouncing gaits (Geyer et al., 2003). Additionally, if the perturbation causes the joints of the limb to extend beyond their normal range prior to landing, muscle stretch, joint proprioceptive and nociceptive responses could inhibit muscle activity (e.g. Gentle, 1992; Gentle et al., 2001; Pearson et al., 1998). Therefore, both muscle preflexes and proprioceptive feedback likely contribute to the reduction in $F_{g,mean}$. Consequently, a more detailed analysis of limb mechanics with simultaneous recordings of muscle force and electromyographic (EMG) activity will be necessary to assess the relative importance of each.

What causes the variation in energy exchange response during the perturbation? The frequency of 'E_{Kh}' mode *vs* 'E_{com}' mode does not relate to the animal's velocity ($V_{i,h}$) or its size. One possible explanation is varied proprioceptive feedback and resulting reflex action due to different limb loading during the tissue break-through phase of the perturbation. It is certainly likely that tissue breakthrough provided proprioceptive feedback that may have influenced the animal's subsequent motor response. Since we could not measure the breaking force of the tissue paper, we cannot address this issue directly. However, if a difference in reflex action distinguished these two responses, one might expect a difference in force development or limb cycle timing. Yet, neither t_c nor |**J**| differs between them (Fig. 6). Although there were no obvious kinematic or behavioral differences prior to tissue paper contact, even slight variation in landing velocity, limb positioning or breaking force of the tissue paper could alter limb extension or limb angle at ground contact, subsequently influencing the intrinsic dynamics of the response. In contrast, the 'E_{Kv}' response shows a dramatic decrease in |**J**| and t_c (Fig. 6), which could be related to a different reflex action during tissue break through or when one or more of the joints have reached a fully extended position.

Although only the smallest bird exhibited the 'E_{Kv}' response, we observed a continuum of body weight support across all birds during the perturbed step (Fig. 8) that may depend on the balance of proprioceptive feedback from a number of different sources. In the extreme trials that fit into the 'E_{Kv}' category, **J** resembles that of an initial limb impact without the subsequent body loading normally responsible for most of the impulse (Fig. 7D; e.g. McMahon et al., 1987). Unless a correspondingly dramatic change in intrinsic mechanics occurs due to changes in gearing or muscle preflexes, which seems unlikely, this drop in force generation must result from a reflex response inhibiting the limb extensors. In summary, we hypothesize that intrinsic mechanics play a larger role in the energy production or absorption by the limb (Fig. 8B; perturbation energy ratio which distinguishes 'E_{com}' from 'E_{Kh}' mode), whereas proprioceptive feedback contributes substantially to the level of body weight support (Fig. 8A; E_V ratio).

Stabilization during hidden vs visible substrate height perturbations

Our second aim is to compare the mechanical response between unexpected *vs* visible perturbations. As mentioned earlier, the birds stumbled, fell, or stopped completely in 50% of V trials. Yet they also maintained a steady spring-like COM trajectory in 10% of the V trials; something they did not accomplish in a single U perturbation. In general, however, guinea fowl were not substantially more successful in preventing a loss of E_P during V trials than during U trials (Fig. 5). Nonetheless, important differences exist between the two conditions. During V perturbations, the birds generally absorbed more energy and exhibited larger impulse magnitudes. Consequently, they were less successful in maintaining forward speed during V drops, but more successful in supporting body weight, resulting in smaller ΔE_{Kv} (Fig. 9). In the 'E_{com}' response among the U drops, the bird absorbed a similar fraction of the ΔE_P through $-\Delta E_{com}$ (Fig. 9). Nonetheless, the 'E_{com}' response exhibited a greater ΔE_{Kv} than V steps. Therefore, the most consistent difference between the perturbation conditions is that all U responses result in lower |**J**| and larger ΔE_{Kv} than V responses (Fig. 9). This suggests feed-forward adjustment of weight support in V trials. When they are able to see and anticipate the upcoming step, the birds maintain weight support and prevent an increase in E_{Kv}, even if it requires losing energy and slowing down (Fig. 9). Although this response may entail a greater energy loss, it might reduce the likelihood of a catastrophic fall.

Recently, Moritz and Farley (2004) found that intrinsic changes in limb mechanics allow hopping humans to be equally successful in maintaining their COM trajectory in response to both expected and unexpected changes in substrate stiffness. Conversely, running guinea fowl show substantially altered COM trajectory and dynamics, whether or not they anticipate the perturbation. There are a couple of possible reasons for the difference between humans and birds in these two studies. First, the relative magnitude of the perturbation may be greater for the guinea fowl, exceeding the capacity for the limb to compensate. Yet, although the ΔH was large, it did not exceed their ability to maintain dynamic stability, as the birds rarely stumbled or fell in the unexpected drops. Furthermore, the birds successfully maintained a steady spring-like trajectory in two of the V drops. An alternative explanation is the difference in mechanics between hopping in place and running; although both are spring-like bouncing motions, running involves retraction of the leg for forward progression. The altered coupling between limb retraction and leg-spring loading may influence COM mechanics more than limb stiffness in the type of perturbation studied here.

Conclusions

Despite large changes in COM dynamics and considerable variability in the response to an unexpected substrate drop, guinea fowl are quite successful in maintaining dynamic stability. The energy exchange patterns show that changes in muscular work play an important role in the dynamics in addition to elastic mechanisms, and suggest altered coupling between limb retraction and limb loading/weight support as an important factor in the mechanical response. Furthermore, the magnitude and direction of the GRF impulse are sufficient to distinguish the mechanics along two axes relating to weight support and limb actuation, respectively. The varied mechanical responses suggest rapid joint extension during the perturbation leading to altered limb posture, intrinsic mechanics, and reflex action. Further investigation into the limb mechanics and muscle activity patterns underlying these varied responses could yield further insight into the control mechanisms that allow such robust dynamic stability during running in the face of large, unexpected perturbations.

List of symbols and abbreviations

a_h	fore–aft instantaneous acceleration
a_v	vertical instantaneous acceleration
BW	body weight
C	control trial
COM	center of mass
E_{com}	total COM energy
E_H	horizontal energy
E_K	kinetic energy
E_{Kh}	horizontal kinetic energy
E_{Ktot}	total kinetic energy
E_{Kv}	vertical kinetic energy
EMG	electromyographic
E_P	potential energy
E_V	vertical energy
$F_{g,mean}$	mean force during contact
f_h	instantaneous fore–aft GRF
f_v	instantaneous vertical GRF
g	gravitational acceleration
GRF	ground reaction force
ΔH	drop in substrate height
HH	hip height

J	resultant impulse vector
|**J**|	magnitude of **J**
j_h	fore–aft component of **J**
j_v	vertical component of **J**
k_{leg}	limb stiffness
M_b	body mass
MSE	mean square error
s_h, s_v	instantaneous position
$S_{i,h}$, $S_{i,v}$	initial positions
SSD	sum of the squared differences
t_c	ground contact time
THSD	Tukey Honestly Significant Difference *post hoc* test
TMP	tarsometatarsophalangeal joint
U	unexpected drop trial
V	visible drop trial
v_h, v_v	instantaneous velocity
$V_{i,h}$, $V_{i,v}$	initial velocity
V$_i$	initial velocity vector
V$_f$	final velocity vector
Σl_{seg}	sum of limb segment lengths
ϕ	angle of **J**, relative to horizontal

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