

Low metabolic cost of locomotion in ornate box turtles, *Terrapene ornata*

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SUMMARY

Evolution has produced a wide range of body plans, but for a given body mass, the energetic cost of transport (COT) of terrestrial animals falls in a relatively narrow range. Previous research indicates that the COT depends on the proficiency of minimizing mechanical work performed, efficiency of performing that work, and cost of generating force to support weight. Turtles are unique in that their protective shell and shoulder-girdle articulation may eliminate the need for the ‘muscular sling’. In addition, turtles have slower, more efficient muscles than other vertebrates. However, slow locomotion may raise the COT by confounding mechanical-energy conservation *via* the inverted-pendulum mechanism. Our goal was to determine the metabolic COT and efficiency of a terrestrial turtle species during locomotion. We studied 18 ornate box turtles, *Terrapene ornata*. Walking speed was extremely slow ($0.07 \pm 0.005 \text{ m s}^{-1}$). The average minimum COT was $8.0 \pm 0.70 \text{ J kg}^{-1} \text{ m}^{-1}$ attained at $\sim 0.1 \text{ m s}^{-1}$. Ornate box turtles consume only half the energy predicted by the allometric relationship for all terrestrial animals ($15.9 \pm 0.35 \text{ J kg}^{-1} \text{ m}^{-1}$), and, thus, appear to be very economical walkers. When walking up a 24 deg. incline turtles moved significantly slower ($0.04 \pm 0.004 \text{ m s}^{-1}$), but performed the extra work required to walk uphill with very high efficiencies (>49%). It appears that the co-evolution of a protective shell, the associated shoulder morphology, and very slow, efficient muscles produce both economical level walking and efficient uphill walking.

Key words: biomechanics, locomotion, energetic cost of transport, muscular efficiency, *Terrapene*.

INTRODUCTION

The energetic cost of transport (COT) for terrestrial locomotion has been of interest to physiologists for many years, and numerous studies have compared the metabolic COT between different taxa (Schmidt-Nielsen, 1972; Taylor et al., 1982; Bennett, 1985; Full, 1989). Previous research has suggested that the energetic COT largely depends on (1) the proficiency of minimizing the mechanical work performed (Cavagna et al., 1977), (2) the efficiency of performing that mechanical work (Taylor, 1994), and (3) the cost of generating force to support body weight (Kram and Taylor, 1990; Griffin et al., 2003). Long-term evolutionary processes have resulted in a wide range of body plans, yet for a given body mass, metabolic COT converges to a relatively narrow range. New insights have come from studies of ‘outliers’ from the allometrically predicted COT (i.e. animals with unusual morphology and/or gaits) such as kangaroos (Dawson and Taylor, 1973), penguins (Pinshow et al., 1977; Griffin and Kram, 2000), platypuses (Fish et al., 2001) and turtles (Baudinette et al., 2000).

Chelonians (turtles and tortoises) have unique anatomical and physiological traits and obviously are slow moving. All chelonians have a protective shell and a specialized articulation between the dorsal prong of the endoskeletal girdle (scapula) and the carapace (top shell) (Walker, 1986). This articulation along with the presence of a plastron (bottom shell) may eliminate the need for the ‘muscular sling’ that is required by other quadrupeds (e.g. Carrier et al., 2006), thus possibly saving energy. In addition, Woledge (Woledge, 1968) demonstrated experimentally that tortoise muscle (*in vitro*) is much slower and much more efficient (35%) than the muscles of other vertebrates (e.g. frog, 20%). Nwoye and Goldspink have shown that the biochemical efficiency of muscle is inversely related to its shortening speed (Nwoye and Goldspink, 1981) and slow-twitch oxidative fibers also require less energy to generate isometric force

than fast-twitch muscle fibers (Heglund and Cavagna, 1985). At the whole-organism level, Baudinette and colleagues showed that Murray short-necked turtles (*Emydura macquarii*) use roughly half as much metabolic energy to walk on the level as other animals of similar size (Baudinette et al., 2000). Thus, the extremely slow rate of movement and high muscular efficiency of chelonians appears to save metabolic energy during terrestrial locomotion. However, of the over 250 turtle species, this relationship has been studied in only one (Baudinette et al., 2000). Furthermore, that species, *Emydura macquarii*, is a semi-aquatic turtle and not a terrestrial specialist. These facts led us to investigate further the energetics of turtle locomotion.

Although slow locomotion with efficient muscles can lead to low COT, slow walking may conversely increase the COT by confounding mechanical-energy conservation *via* the inverted-pendulum mechanism. Cavagna and colleagues (Cavagna et al., 1977) demonstrated in many species that the inverted-pendulum mechanism of mechanical-energy conservation during walking is greatest at intermediate speeds. In addition, several recent studies have revealed that extremely slow animals, such as walking alligators and giant tortoises, have only low levels of energy conservation utilizing the inverted-pendulum mechanism (Willey et al., 2004; Zani et al., 2005). Thus, the physiological efficiency benefits of using slow muscle fibers may be offset by poor mechanical-energy conservation that results in greater mechanical work. In this study, our goal was to quantify the metabolic energy expenditure during locomotion (i.e. *in vivo*) and thus calculate metabolic COT and locomotor efficiency of a terrestrial species of turtle, the ornate box turtle (*Terrapene ornata*). We hypothesized that *Terrapene ornata* would have a lower COT during level walking than that predicted based on body mass (Taylor et al., 1982; Full, 1989) and a greater uphill walking efficiency than has been measured for other species.

MATERIALS AND METHODS

Ornate box turtles (*Terrapene ornata*, Agassiz 1857; average mass \pm 1 s.d., 0.310 ± 0.0867 kg; range, 0.173–0.431 kg) were collected in the vicinity of Hays, KS, USA in the spring of 2003 and transported to the University of Colorado in Boulder, CO, USA. Turtles were housed in 1 m diameter circular cages with 10 cm of sand depth as substrate and provided with shelter, water and heat (60 W full-spectrum incandescent bulbs). In addition, the room was lit by eight fluorescent bulbs (light:dark, 18 h:6 h). We fed turtles a variety of fruits (e.g. watermelon, pumpkin, orange, banana, apple) mixed with ground dry dog food (Science Diet large-breed puppy chow) or wet cat food (9 Lives) every 2 days. In addition, we occasionally fed turtles earthworms, wax worms, pinkie rats, and crickets dusted with multivitamin. Turtles were not fed for at least 48 h prior to data collection.

From mid-December 2003 to late-January 2004, turtles were maintained in a refrigerator in a state of cold torpor at 5°C in 1 gallon (~ 3.81) plastic boxes (two per box) partially filled with moist soil. During the last 2 weeks of this 6 week period, the daily fluctuation in thermoperiod was gradually increased by $\sim 5^{\circ}\text{C}$ to mimic spring-like conditions. Turtles were then placed back into their bins and provided with heat, food and water. The light:dark cycle was initially set at 14 h:10 h, and day length was increased by 15 min per week over the course of data collection to simulate spring. All turtles survived and only individuals that appeared to be healthy were included in this study.

We trained turtles to walk on a motorized treadmill with a belt that was coated with sand to provide traction (bed dimensions 0.15 m wide \times 0.5 m long). Turtles were encouraged to walk continuously by a researcher tapping on the shell with a pencil. Turtles wore a loose-fitting cylindrical acetate open-flow mask (Fig. 1; attached to the shell with Velcro) from which expired air was dried with Drierite (W. A. Hammond Drierite Co. Ltd, Xenia, OH, USA) and then drawn through an oxygen analyzer (S3A Ametek, Paoli, PA, USA). The appropriate flow rate was determined by calculating oxygen consumption during a sample of duplicate walking trials at several flow rates (1.0, 0.5, 0.4 and 0.31 min^{-1}). For all subsequent walking trials, we used the lowest flow rate (0.41 min^{-1}) that resulted in an identical metabolic rate to that for the faster flows. Data were recorded using LabView 6.1 for Macintosh. Oxygen consumption was calculated using the formulas of Bartholomew and colleagues (Bartholomew et al., 1981). Rates of oxygen consumption were converted to rates of energy consumption (i.e. metabolic rates) by assuming 20.1 J of energy are released for each milliliter of oxygen consumed. A total of 268 trials were performed on 18 individual box turtles at room temperature ($22.9 \pm 1.99^{\circ}\text{C}$) over 3 months. Each

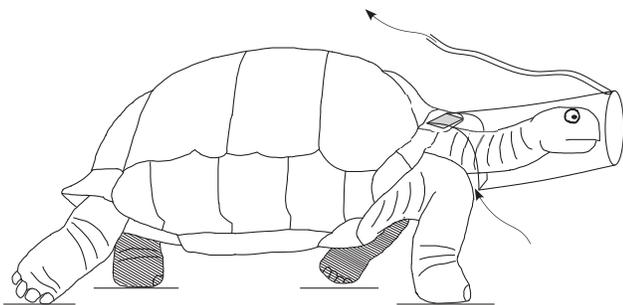


Fig. 1. Drawing of the open-flow acetate mask worn by turtles. Arrows indicate the direction of air flow. The mask was attached by Velcro to the shell, but otherwise did not touch the animal during locomotion.

trial began with a 5–8 min accommodation period that allowed animals to reach steady-state metabolism. During this period, animals were required to walk on the treadmill as normal. We considered turtles to have reached steady-state metabolism when their rate of oxygen consumption leveled off for at least 1 min. Following this, we calculated oxygen consumption over a 4 min period of level walking during which time the metabolic rate was relatively unchanged. We were able to collect oxygen consumption data for 4 min of steady walking for up to 10 trials per individual. Following a successful level trial, we raised the front end of the treadmill so that it was inclined 24° . This angle was chosen because it is near the steepest incline that turtles could still walk. After an additional 4–6 min accommodation, we measured the average oxygen consumption over a 4 min period for incline walking. Following each trial, we graded each part (level, incline) as ‘excellent’, ‘good’ or ‘poor’ based on turtle cooperativeness during the period of accommodation and data collection. A trial in which the turtle rarely stopped or stopped only briefly was denoted as excellent; if a turtle stopped occasionally or stopped for no longer than 5 s at a time, it was rated as good; if during a trial a turtle stopped frequently for long time periods or turned around on the treadmill, the trial was deemed poor.

Cost of transport

To estimate the minimum cost of transport (COT_{min}), we used three methods. One standard way to compare the expense of locomotion across taxa is to represent the metabolic rate for locomotion as a function of speed (e.g. Taylor et al., 1982). The slope of the relationship between metabolic rate and speed is known as the energetic COT ($\text{J kg}^{-1} \text{ m}^{-1}$) and represents the amount of energy required to move a given body mass a given distance. However, the range of steady speeds obtained during this study was slow and narrow (0.116 m s^{-1}) and presented a new challenge for determining COT_{min} . COT can also be calculated for a single trial by dividing metabolic rate by speed. COT_{min} can then be determined as the minimum of the curvilinear regression of individual data points for COT versus speed. Since this includes all trials from all individuals this probably represents a conservative estimate of COT_{min} . A third way of calculating COT_{min} is to select the lowest value of COT for all trials for each individual and then averaging these values for all individuals. Because turtles were not able/willing to sustain speeds that might have further minimized energy consumption, each of these methods may overestimate COT_{min} . However, we feel that these methods provide the best possible options for calculating COT_{min} .

Locomotor efficiency

To lift and accelerate the center of mass of the body and to move the limbs relative to the body during each stride, legged animals must perform some amount of mechanical work. Yet, there is no consensus among biomechanists on how to measure this work (e.g. Willems et al., 1995; Schenau, 1998; Zatsiorsky, 1998; Donelan et al., 2002; Bastien et al., 2003). However, uphill locomotion involves an unambiguous increase in the work required to steadily lift the center of mass against the force of gravity. By comparing level and incline walking, we were able to estimate locomotor efficiency. Locomotor efficiency was determined by first calculating the extra mechanical power output required on the incline as $mgv\sin\theta$, where m is body mass (kg), g is 9.81 m s^{-2} , v is belt speed (m s^{-1}) and θ is incline angle (deg.). For example, at a speed of 0.04 m s^{-1} on an incline, for the average mass of our animals (0.31 kg), mechanical power was $0.31 \times 9.81 \times 0.04 \times \sin 24 = 0.049 \text{ W}$. Following this, the metabolic power was calculated by multiplying the difference in

the mass-specific rates of oxygen consumption ($\text{ml O}_2 \text{ kg}^{-1} \text{ s}^{-1}$) for incline and level walking by $20.1 \text{ J ml O}_2^{-1}$ and mass (kg). Efficiency was then calculated as the ratio of mechanical-power output to metabolic-power input. For example, at 0.04 m s^{-1} , if oxygen consumption on the level was $0.040 \text{ ml O}_2 \text{ kg}^{-1} \text{ s}^{-1}$ and for uphill walking it was $0.054 \text{ ml O}_2 \text{ kg}^{-1} \text{ s}^{-1}$ there is a difference of $0.014 \text{ ml O}_2 \text{ kg}^{-1} \text{ s}^{-1}$. This, multiplied by $20.1 \text{ J ml O}_2^{-1}$ and a mass of 0.31 kg , results in an increased metabolic power of 0.087 W . Thus, at 0.04 m s^{-1} the ratio of mechanical to metabolic power is $0.049 \text{ W} / 0.087 \text{ W} = 0.563$ for a locomotor efficiency of 56.3%.

Resting metabolism

As an additional comparison, we conducted two resting metabolism trials on each individual. Each turtle was handled as usual and placed on the treadmill while wearing the open-flow mask and allowed to remain still for 5 min. Animals were unrestrained during this period, but if they started to move we would hold a hand in front of their heads or rap once on their shells with a pencil. This had the effect of startling the turtle's head into its shell slightly and ceasing its attempt to move. However, excessive startling could result in the animal completely withdrawing its head into its shell (and out of the mask). Thus, care was taken to ensure that the animal neither moved excessively nor withdrew its head from the mask for at least four consecutive minutes during a 15 min trial.

RESULTS

We were able to collect 141 walking trials graded as excellent from the terrestrial *T. ornata*. Only excellent trials were included in subsequent analyses. Turtles walked continuously on a level treadmill over a relatively small range of speeds ($0.018\text{--}0.134 \text{ m s}^{-1}$; average $\pm 1 \text{ s.d.}$, $0.07 \pm 0.005 \text{ m s}^{-1}$). The average rate of oxygen consumption during all excellent level trials was $0.044 \pm 0.0170 \text{ ml kg}^{-1} \text{ s}^{-1}$ ($0.158 \pm 0.0611 \text{ ml g}^{-1} \text{ h}^{-1}$). The rate of oxygen consumption during level walking increased with locomotor speed (Fig. 2A; $F_{1,139} = 9.45$; $P = 0.003$). However, variation in speed only explained 6.4% of the variation in oxygen consumption during level locomotion.

From these metabolic rate data, we estimated level COT_{min} in three ways. First, we simply used the slope of the metabolic rate versus speed and multiplied by $20.1 \text{ J ml O}_2^{-1}$ (Fig. 2A). The slope ($\pm 1 \text{ s.d.}$) of this relationship was $0.226 \pm 0.0734 \text{ ml O}_2 \text{ kg}^{-1} \text{ m}^{-1}$, which equates to a COT_{min} of $4.5 \text{ J kg}^{-1} \text{ m}^{-1}$. We also calculated the 95% confidence intervals for this slope (Zar, 1999) as $0.01\text{--}0.45 \text{ ml O}_2 \text{ kg}^{-1} \text{ m}^{-1}$. Using the upper (most conservative) estimate for slope would produce a COT of $9.07 \text{ J kg}^{-1} \text{ m}^{-1}$. Second, we calculated the COT of each trial from metabolic rate data (Fig. 2B). Based on a repeated-measures ANOVA on the 18 turtles with at least eight trials over 3 months, the COT of turtles walking on the level did not change significantly with training ($F_{7,77} = 1.91$; $P = 0.080$). If anything, the trend was toward a slightly increased COT with trial date. When plotted against speed, it is apparent that the COT decreased as expected. A second-order polynomial provided the best curve fit to these data, explaining nearly 23% of the variation in COT (versus only 18% for the linear regression). By setting the first derivative of the regression equation equal to zero, we determined that the minimum COT occurred at 0.109 m s^{-1} . The COT at this speed was estimated to be $9.8 \text{ J kg}^{-1} \text{ m}^{-1}$ with 95% confidence intervals (at this minimum speed) of 5.1 to $14.5 \text{ J kg}^{-1} \text{ m}^{-1}$. However, in only three of 141 included trials did turtles walk faster than 0.11 m s^{-1} making it difficult to verify that this was actually the minimum COT . Third, we determined COT_{min} for each of the 18 individuals simply as their lowest COT trial

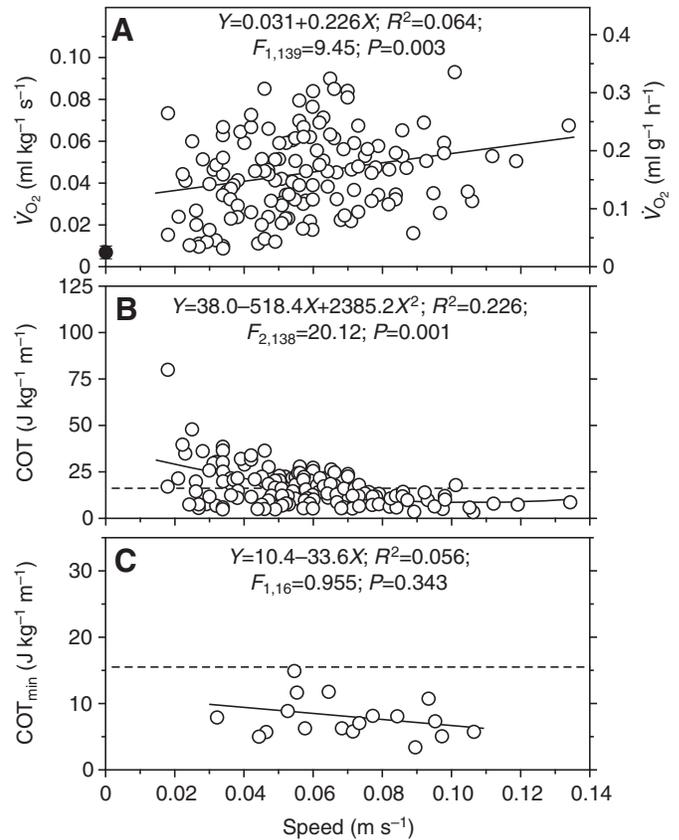


Fig. 2. Data from 141 trials of 18 individual *Terrapene ornata* walking on the level showing (A) rate of oxygen consumption versus speed for all trials, (B) cost of transport (COT) versus speed for all trials, and (C) minimum COT (COT_{min}) for up to 10 trials for each individual versus speed. Dashed line indicates the expected COT based on mass (Full, 1989). Average resting \dot{V}_{O_2} ($\pm 1 \text{ s.d.}$) is shown as a filled circle at 0 m s^{-1} .

(Fig. 2C). The average of the 18 COT_{min} values was $8.0 \pm 0.70 \text{ J kg}^{-1} \text{ m}^{-1}$ with 95% confidence intervals of 6.31 to $9.69 \text{ J kg}^{-1} \text{ m}^{-1}$.

When walking up a 24 deg. incline compared with level walking, *T. ornata* showed a significant decrease in walking speed (level: $0.07 \pm 0.005 \text{ m s}^{-1}$; incline: $0.04 \pm 0.004 \text{ m s}^{-1}$; repeated-measures ANOVA on average speed for each turtle: $F_{1,17} = 22.4$; $P < 0.001$). By limiting data analysis to the range of speeds common to level and incline locomotion (i.e. $0.018\text{--}0.062 \text{ m s}^{-1}$) and pairing trials in which turtles walked at the same speed ($\pm 0.005 \text{ m s}^{-1}$), we were able to determine the effects of walking on an incline on metabolic rate and COT (Fig. 3). The metabolic rate of turtles on an incline [Fig. 3A; all trials ($N = 108$): $0.051 \pm 0.0223 \text{ ml kg}^{-1} \text{ s}^{-1}$] was significantly greater than on the level (repeated-measures ANOVA on turtles with trials matched for speed: $F_{1,17} = 8.41$; $P = 0.010$). Likewise, the COT of turtles on an incline (Fig. 3B,C; all trials: $15.0 \pm 1.67 \text{ J kg}^{-1} \text{ m}^{-1}$) was significantly greater than on the level (repeated-measures ANOVA on turtles with trials matched for speed: $F_{1,17} = 4.98$; $P = 0.039$). However, because variation in the rate of oxygen consumption was so great (Fig. 2A; Fig. 3A), we calculated whole-animal locomotor efficiency (ratio of mechanical to metabolic powers) for the species as a whole by integrating over the range of speeds in common ($0.018\text{--}0.062 \text{ m s}^{-1}$) at 0.0001 m s^{-1} increments. The average locomotor efficiency for ornate box turtles over this range of speeds was 59.6% (range, 49.3% at 0.062 m s^{-1} ; 96.7% at 0.018 m s^{-1}).

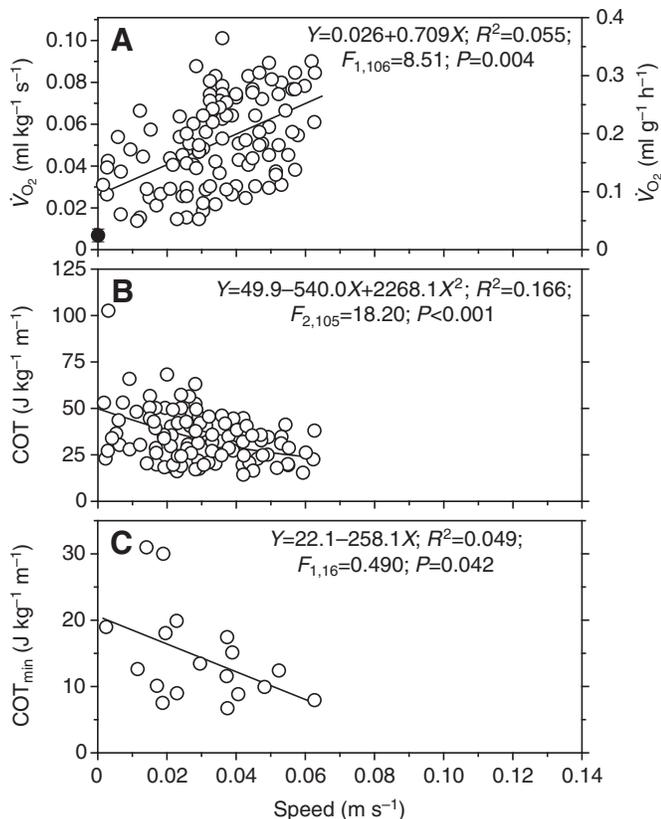


Fig. 3. Data from 108 trials of 18 individual *Terrapene ornata* walking on a 24.1 deg. incline showing (A) rate of oxygen consumption versus speed for all trials, (B) COT versus speed for all trials, and (C) COT_{min} for up to 10 trials for each individual versus speed. Dashed line is expected COT based on mass (Full, 1989). Note, horizontal axis is the same scale as in Fig. 2. Average resting \dot{V}_{O_2} (± 1 s.d.) is shown as a filled circle at 0 m s⁻¹.

The average rate of oxygen consumption of turtles resting on the treadmill, but not moving, was 0.006 ± 0.0009 ml kg⁻¹ s⁻¹.

DISCUSSION

As hypothesized, ornate box turtles (*Terrapene ornata*) have a very low metabolic COT during level walking. Our three estimates for COT_{min} (based on linear slope of the metabolic relationship to speed, quadratic minimization of COT calculated for each trial, and average of minimum COT values for each individual) were 4.5, 9.8 and 8.0 J kg⁻¹ m⁻¹, respectively. The upper estimates from the 95% confidence intervals were 9.07, 14.5, 9.69 J kg⁻¹ m⁻¹, respectively. Values obtained with the slope method present some confusion since it overlooks the basal or resting metabolic rate. For active endotherms, this is not a big problem, but as is obvious from Fig. 2A the walking metabolic rates were often barely above the resting metabolic rate. For this reason, we are not proponents of the slope method for our turtle data. The second method, based on the polynomial regression equation for COT for all trial values (Fig. 2B), is likely to give a conservative estimate since it includes multiple trials per individual and, thus, incorporates the variation in oxygen consumption rates apparent in Fig. 2A. We consider the third method, the average of minimum values for each individual (Fig. 2C), to be a better estimate of COT_{min} than either of the two alternatives. Regardless of the method used, the COT of box turtles is substantially lower than the expected COT (15.9 ± 0.35 J kg⁻¹ m⁻¹)

using the allometric relationship for all animals, including other reptiles (Full, 1989). Even the upper tail of the 95% confidence interval of our most conservative estimate of COT_{min} (14.5 J kg⁻¹ m⁻¹) is below the estimate for this species based on its size.

This is only the second report of the COT of terrestrial locomotion of a chelonian and the first report for a terrestrial specialist. Baudinette and colleagues studied a semi-aquatic species of turtle (walking at near the same speeds as box turtles) and found that the COT (5.97 J kg⁻¹ m⁻¹) was roughly half that expected (12.55 J kg⁻¹ m⁻¹) based on mass (Baudinette et al., 2000). In addition to calculating the expected COT of box turtles using the allometric relationship of Full (Full, 1989), we used measurements of COT for lizards reviewed most recently by Hare and colleagues (Hare et al., 2007) to compare the COT of chelonians with those of other reptiles (see also John-Alder et al., 1986; Autumn et al., 1999). By including the 23 species reviewed by Hare and colleagues (Hare et al., 2007), the estimate for *Emydura* (Baudinette et al., 2000), and our best estimate of COT (8.0 ± 0.70 J kg⁻¹ m⁻¹; Fig. 4), we determined that both species of chelonians studied thus far have among the lowest metabolic COT. These costs are similar to those of many nocturnal lizards (e.g. Autumn et al., 1999; Hare et al., 2007). Both species of turtle fall outside the 95% confidence intervals, which supports the notion that among reptiles, turtles have a low energetic expenditure during locomotion.

There are several possible explanations for the low COT in turtles. First, turtles are unique among vertebrates in that they have a moveable joint (articulation) between the dorsal prong of the endoskeletal girdle (scapula) and the carapace (top shell). The cranioventral prong of the endoskeletal girdle (acromion) attaches to the plastron (lower shell) via a ligament. Along with the presence of a bottom shell, this articulation may minimize the need for a 'muscular sling' (*sensu* Carrier et al., 2006) to support the weight of the front part of the body, potentially providing an energetic saving during locomotion. The evolutionary changes in shoulder-girdle articulation appear gradually in the protochelonian fossil record [see figure 12 of Lee (Lee, 1997)] and are nearly indistinguishable from extant forms even in the earliest chelonian fossils [e.g. 210 mya *Proganochelys quenstedti* [American Museum of Natural History (AMNH) no. 29383]; P.A.Z. unpublished observation] suggesting that these innovations evolved early in the turtle lineage and have changed very little since.

A second possible explanation for the low metabolic COT of turtles relates to their muscle physiology. Woledge (Woledge, 1968)

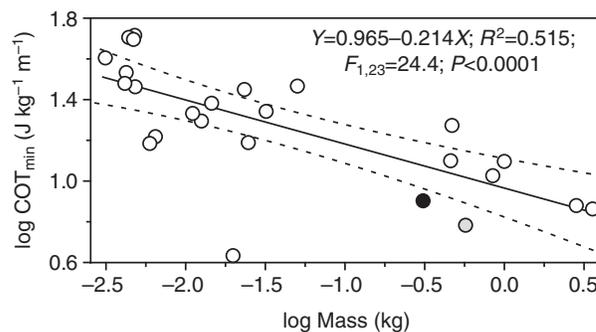


Fig. 4. Log-log plot of body mass versus COT_{min} from 25 reptiles. Open circles are 23 species of lizard (reviewed by Hare et al., 2007). Gray circle is Murray short-necked turtle (Baudinette et al., 2000). Black circle is ornate box turtle (this study). Solid trend line is least-squares regression. Dashed lines are 95% confidence intervals.

reported that turtle muscles are more efficient (35%) *in vitro* than those of other vertebrates (e.g. frog, 20%). This suggested that turtles would be more efficient at moving. When we compared the metabolic cost of level with that of uphill locomotion we estimated the *in vivo* efficiency of turtles to be at least 49% and averaging 59.6% over the entire walking speed range. In addition to calculating efficiency over the range of speeds common to level and incline locomotion, we extrapolated our efficiency calculations to the fastest sustainable speed that we measured for this species (0.134 m s^{-1}) as well as burst speeds for this species (0.208 m s^{-1}) reported by Claussen and colleagues (Claussen et al., 2002) and found locomotor efficiencies of 44.5% and 43.3%, respectively. We surveyed the literature for reports of the metabolic cost of level and uphill locomotion to compute locomotor efficiency in other species (see Materials and methods). Indeed, turtles appear to be unusually efficient when compared with other animals (Table 1). This high efficiency could be due, in part, to the fact that muscular efficiency is inversely related to the shortening speed of muscles (Nwoye and Goldspink, 1981).

When trying to understand the low COT in turtles, it may be useful to consider further the potential co-evolution of slow speed and a protective shell. Lovegrove (Lovegrove, 2001) related the evolution of body armor in mammals to basal metabolic rates and locomotor performance (running speeds). Following the ideas of Lovegrove (Lovegrove, 2001), we hypothesize that the evolution of effective defenses (e.g. dermal plates, shells, spines, noxious chemicals) may release animals from selection on enhanced locomotor speed performance for escape, resulting in slower walking/running speeds and, hence, energetic savings due to the inverse relationship between speed and muscular efficiency. In other words, an animal with an effective defense need not be a fast locomotor and, thus, defenses and metabolic economy may co-evolve. A test of this hypothesis might include a comparative study of related species with and without defenses (e.g. skunks with other mustelids; porcupines with other rodents) to determine whether species with defenses are slow but economical locomotors in general.

A third explanation for the low energetic COT could be excellent conservation of mechanical energy using mechanisms such as inverted-pendulum energy exchange. Species with lower metabolic COT would be expected to have more proficient exchange of kinetic and gravitational potential energies during walking. However, the

only completed study of the inverted-pendulum mechanism in chelonian locomotion found that Galapagos tortoises have much greater potential energy fluctuations than kinetic energy fluctuations when they walk and thus their inverted-pendulum energy exchange is poor (Zani et al., 2005). Preliminary analysis suggests this pattern is similar in ornate box turtles (Zani et al., 2004) (P.A.Z., R.K., A. R. Biknevicus and S. M. Reilly, unpublished data). Willey and colleagues reported very little mechanical-energy recovery for alligators, which are similarly slow (Willey et al., 2004). Extremely slow locomotion apparently precludes effective inverted-pendulum exchange during walking because there is too little kinetic energy available to convert into gravitational potential energy. Thus, chelonians conform to the definition of 'lumbering' (*sensu* Reilly et al., 2006) in which fluctuations in potential energy are much greater than fluctuations in kinetic energy (see also Reilly et al., 2007). While evolutionarily correlated with the presence of a shell, it is unclear whether the shell necessarily caused this transition in chelonians.

The active and resting oxygen consumption rates we calculated for ornate box turtles are similar to those of a previous report for this species. At 23°C Gatten (Gatten, 1974) reported that the average active oxygen consumption rate was $\sim 0.083\text{ ml kg}^{-1}\text{ s}^{-1}$ while the resting rate was $\sim 0.003\text{ ml kg}^{-1}\text{ s}^{-1}$. Our active and resting oxygen consumption rates were 0.044 and $0.006\text{ ml kg}^{-1}\text{ s}^{-1}$, respectively. We ascribe these differences to the fact that we studied turtles walking on a treadmill at sustainable, aerobic speeds whereas Gatten (Gatten, 1974) induced maximal activity by several minutes of electric shock. Similar to the findings of Gatten (Gatten, 1974), our data exhibit considerable variation in the rate of oxygen consumption (Fig. 2A). We considered several possible explanations for this variation. First, turtles may have relied on anaerobic metabolism during locomotion. To test this possibility, we conducted several trials in which animals were allowed to rest after an extended period ($\sim 15\text{ min}$) of level walking. In all cases, the rate of oxygen consumption dropped to near resting within 20–30 s indicating that the turtles had not relied on anaerobic metabolism while walking. Second, turtles may have been holding their breath intermittently during locomotion. Early in this study, we noted this behavior in one individual that held his breath for $\sim 15\text{ min}$ while walking continuously and only breathed when he stopped. Interestingly, the total energy consumed for that trial was similar to trials in which animals breathed normally (i.e. when the turtle stopped and breathed

Table 1. Locomotor efficiencies calculated from literature reports of metabolic cost of level *versus* uphill walking/running

| Species | Mass (kg) | Incline (deg.) | Efficiency (%) | Reference |
|-------------|-----------|----------------|----------------|-----------------------------|
| Turtles | 0.316 | 24 | 59.6 | This study |
| Geckos | 0.0042 | 50 | 37.0 | Farley and Emshwiller, 1996 |
| Skinks | 0.0048 | 50 | 19.3 | Farley and Emshwiller, 1996 |
| Cockroaches | 0.0008 | 45 | 4.4 | Full and Tullis, 1990 |
| | | 90 | 3.4 | |
| Mice | 0.030 | 15 | 60 | Taylor et al., 1972 |
| Rats | 0.225 | 16 | 23 | Armstrong et al., 1983 |
| Squirrels | 0.250 | 6 | 11 | Wunder and Morrison, 1974 |
| | | 18 | 41 | |
| | | 30 | 35 | |
| | | 37 | 30 | |
| Dogs | 12.8 | 4.23 | 41.8 | Raab et al., 1976 |
| | | 6.7 | 36.9 | |
| | | 11.5 | 30.4 | |
| Kangaroos | 20.4 | 7.9 | 30.0 | Kram and Dawson, 1998 |
| | | 11.7 | 28.8 | |
| | | 14 | 30.7 | |
| Chimpanzees | 17.5 | 15 | 66 | Taylor et al., 1972 |

his oxygen consumption spiked, but averaged over the period of walking metabolic rate was 'normal'). After noting this breath-holding behavior, we carefully monitored trials for signs of breath holding in other turtles (e.g. dips and spikes in oxygen consumption). All other individuals appeared to breath normally throughout all trials and that one individual was excluded from further study. Third, turtles are sometimes clumsy in their movements and will misstep, landing on their shells before continuing to walk. This did not appear to impede their locomotion as turtles were able to recover immediately and is akin to a 'stumble' in humans. Yet this may have made the locomotion of some individuals prone to stumbling energetically less efficient. However, at best we can note that Gatten (Gatten, 1974) had a similar spread in the rate of oxygen consumption at a given temperature for this same species [see figure 2 of Gatten (Gatten 1974)] and that individual variation may lead to variation in rates of oxygen consumption and the subsequent determination of COT (Fig.2C). Thus, we have no obvious explanation for the variation in the rate of oxygen consumption, except that metabolic rate appears to vary from trial to trial within what we considered to be excellent trials. Rather than obscure the variation by only reporting average values for each individual, we elected to plot all data points from all excellent trials.

In summary, ornate box turtles walk extremely slowly, and with very low metabolic costs relative to their body size. Furthermore, the uphill locomotion of ornate box turtles appears to be unusually efficient (>49%) compared with that of most other vertebrates. Since the only other turtle species studied to date revealed poor mechanical-energy recovery using the inverted-pendulum mechanism (~30%), the low metabolic COT in turtles appears to be due to the morphological changes that accompanied the evolution of a protective outer shell, extremely slow locomotor speeds and unusually efficient muscles. It remains to be seen whether the origins of defense mechanisms in other vertebrate lineages resulted in convergent evolutionary changes with respect to locomotor energetics and mechanics.

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