Crouching to fit in: the energetic cost of locomotion in tunnels

Angela M. Horner[1],*, Jandy B. Hanna[2] and Audrone R. Biknevicius[3]

ABSTRACT

Animals that are specialized for a particular habitat or mode of locomotion often demonstrate locomotor efficiency in a focal environment when compared to a generalist species. However, measurements of these focal habitats or behaviors are often difficult or impossible to do in the field. In this study, the energetics and kinematics of simulated tunnel locomotion by two unrelated semi-fossorial mammals, the ferret and degu, were analyzed using open-flow respirometry and digital video. Animals were trained to move inside of normal (unconstrained, overground locomotion) and height-decreased (simulated tunnel, adjusted to tolerance limits for each species) Plexiglas chambers that were mounted flush onto a treadmill. Both absolute and relative tunnel performance differed between the species; ferrets tolerated a tunnel height that forced them to crouch at nearly 25% lower hip height than in an unconstrained condition, whereas degus would not perform on the treadmill past a ~9% reduction in hip height. Both ferrets and degus exhibited significantly higher metabolic rates and cost of transport (CoT) values when moving in the tunnel condition relative to overground locomotion. When comparing CoT values across small (<10 kg) mammals, ferrets demonstrated a lower than predicted metabolic cost during both tunnel and terrestrial locomotion, whereas degus were very close to the line of best fit. Although tunnel locomotion requires a more striking change in posture for ferrets, ferrets are more efficient locomotors in both conditions than mammals of similar mass.

KEY WORDS: Locomotion, Mammal, Energetics, Tunnel, Semi-fossorial, *Mustela*, Ferret, Cost of transport

INTRODUCTION

Many organisms encounter variable terrains in their daily lives that require alterations of locomotor behavior and may incur substantial energetic costs. Locomotor 'specialists' mitigate some of these costs through morphological or physiological adaptations to a focal environment, but often experience the trade-off of performing less well in other environments. For example, the flying squirrel (*Glaucomys sabrinus*) is quite successful at utilizing gliding mechanics to efficiently navigate arboreal habitats but, during terrestrial locomotion, their movement costs greatly exceed that of (non-gliding) fox squirrels (Flaherty et al., 2010). Semi-specialized animals may only have some of the features that their more derived specialist counterparts possess, but

nevertheless must navigate both specialized and terrestrial habitats. Evidence from previous studies suggest that this compromise comes with an energetic cost; for example, the cost of swimming among semi-aquatic mammals (minks and otters) is two- to five-fold greater than for fully aquatic mammals (Williams, 1999). Similarly, although burrowing is an absolutely energetically costly activity (Bozinovic et al., 2005), studies of burrowing energetics in rodents found that burrowing metabolic rates of fossorial specialists tend to be lower than that of semi-fossorial species, suggesting that there are physiological and/or morphological adaptations mitigating some of the costs in burrowing specialists (Bozinovic et al., 2005; Luna and Antinuchi, 2007; White et al., 2006).

Although the act of burrowing is well-known to be energetically demanding, the task of simply moving through tunnels has not yet been investigated. One of the primary physical challenges for limbed animals moving through tunnels is the adoption of a posture to accommodate the body and swing limbs in a constrained environment. In humans, crouched, or 'Groucho', running with bent knees increases the metabolic cost of locomotion by as much as 50% (McMahon et al., 1987). Although the posture of small (<10 kg) mammals is already quite crouched, the fact that burrow entrance holes are sometimes not much bigger than the burrow makers' heads suggests that postural changes are occurring in the tunnel locomotion of many small mammals (Nevo, 1979).

In order to further investigate the potential performance trade-offs of semi-specialization for tunnel locomotion, the energetic cost of moving in tunnels was compared to terrestrial locomotion in a semi-fossorial non-digging musteline, the ferret. Mustelines are known for their distinctive morphologies, with a relatively elongate body shape and short legs that presumably allow these predators to enter tunnels and burrows easily, but still remain capable of overpowering prey that may be as much as two to three times their size (Gambaryan, 1974; King and Powell, 2007). This 'weasel-form' is found in *Mustela* species across the world occupying similar ecological niches (Brown and Lasiewski, 1972; King, 1989; Sandell, 1989), but the physiological effects of this bauplan have previously not been addressed for the supposed adaptive purpose – moving in tunnels. Thus, this study primarily sought to compare the energetic costs of tunnel locomotion to terrestrial locomotion in a semi-fossorial mustelid: the domestic ferret (*Mustela putorius furo*, Linnaeus 1758). In order to explore the potential influences of morphology on performance, we also collected data on a semi-fossorial caviomorph rodent with more generalized morphology, the degu [*Octodon degu* (Molina 1782)]. In this study, we (1) quantify the cost of locomotion in unconstrained and simulated tunnel conditions in two semi-fossorial mammals spanning a fourfold range in size, (2) contrast the kinematics and energetics of unconstrained versus tunnel locomotion within these species, and (3) compare our empirical data to measured and predicted values for similarly sized mammals.

[1]Department of Biology, California State University, San Bernardino, CA 92407, USA. [2]Department of Biomedical Sciences, West Virginia School of Osteopathic Medicine, Lewisburg, WV 24901, USA. [3]Department of Biomedical Sciences, Ohio University College of Osteopathic Medicine, Athens, OH 45701, USA.

*Author for correspondence (anghorner@gmail.com)

A.M.H., 0000-0001-5339-227X

Table 1. Morphological characteristics (means±s.e.m.)

Species	Total body length (cm)	Body mass (g)	Hip height at hindlimb mid-stance (cm)		Peak spine height at hindlimb mid-stance (cm)	
			Unconstrained	Tunnel	Unconstrained	Tunnel
Ferrets (N=4)	36.4±1.4	1034.6±44.1	10.2±0.6	78±0.2	14.0±0.4	9.2±0.1
Degus (N=3)	18.9±0.5	257.9±14.2	4.6±0.4	4.2±0.2	6.3±0.4	5.4±0.3

MATERIALS AND METHODS

Choice of study species and experimental treatments

The domestic ferret (*M. putorius furo*) both resembles and is closely related to the European polecat (*M. putorius*) and the American black-footed ferret (*M. nigripes*). Ferrets are considered to be semi-fossorial as they may forage either above or below ground, but cache food and raise young below ground (King and Powell, 2007). Previous studies have shown that ferrets readily enter tunnels, and there is evidence of some morphological adaptations to accommodate this habitat (Horner and Biknevicius, 2010; Moritz et al., 2007). The degu (*O. degus*) is a South American rodent species that builds extensive networks of burrows in which to sleep and escape predation, but forages aboveground. Thus, both species move in tunnels and fully terrestrial environments.

Morphological data for the animals used in this study are listed in Table 1. The ferrets were obtained through a licensed breeding facility for use in this treadmill-based gait and energetic study and were previously used in a trackway study (Horner and Biknevicius, 2010). The degus were acquired from a colony maintained at Michigan State University. All animals had free access to water and were kept on a 12 h:12 h photoperiod schedule in thermally stable rooms maintained at 21±0.5°C. Before metabolic data were collected, all animals were fasted for 6–8 h to achieve a post-absorptive state. All housing and experimental procedures were approved by Ohio University Institutional Animal Care and Use Committee protocols (U99-03).

Animal training

To ensure behavioral familiarity and similar fitness levels, all animals were trained for a period of at least 3 weeks (degus) to 2 months (ferrets) to run on a motorized treadmill (Jog-A-Dog LLC, Ottawa Lake, MI) within Plexiglas chambers. The chambers were mounted onto an adjustable rack that fit snugly to the treadmill belt. When an individual was able to consistently match a range of treadmill speeds, a series of lowered-height chambers were introduced to simulate tunnel height conditions. Chamber ceilings were incrementally lowered to the minimum height at which all animals could still readily move. As these values were fairly consistent within each species (Table 1), a 'threshold height' was calculated for both and then applied during data collection (Horner and Biknevicius, 2010).

Kinematics and gait variables

For kinematic and gait analyses on the treadmill and for comparison with the overground study, digital video data were recorded at a frame rate of 300 Hz with a Casio Exilim EX-F1 camera mounted in a lateral view. For the ferrets, the timing and order of footfall events were used to define symmetrical gaits following the model of Hildebrand (1976), where gaits are defined by limb phase: the elapsed time between ipsilateral hindlimb and forelimb footfalls divided by total stride cycle duration. Duty factor was calculated by dividing hindlimb support duration by stride duration. Footfall and kinematic data were digitized and analyzed in Peak Motus v. 9.0 software (Vicon). Comparable kinematic datasets were unavailable for the degus because of difficulty in consistently viewing footfalls during the simulated tunnel trials.

Postural differences between unconstrained and height-constrained conditions were assessed using maximum hip and back heights taken at hindlimb midstance in each condition. These distances were calculated by digitizing the vertical position of the hip joint and the thoracolumbar junction (highest vertical position along the back) relative to contact of the left hind foot with the trackway (Fig. 1). Maximum hip height was used to approximate overall limb flexion (i.e. effective limb length), whereas maximum back height reflected the degree of spinal extension occurring owing to height constraints. Individual body lengths were measured from nose tip to the base of tail while the animals were being held, whereas hip heights were measured from videos recording lateral views of the animals on a treadmill.

Metabolic measurements

The rate of oxygen consumption and carbon dioxide production were measured simultaneously with video data using an open flow, TurboFox Flowkit 100 respirometry system (Sable Systems, Las
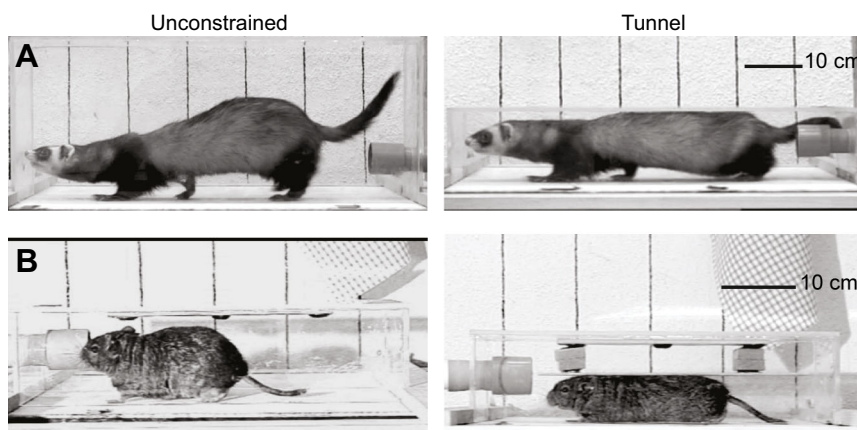

Fig. 1. Digital video stills of ferrets and degus taken during hindlimb midstance in both tunnel and open chamber (epigean) conditions. (A) Ferret. (B) Degu. Within a species, similar speeds were used. Tunnel chambers induced a 24% reduction in hip height in ferrets and a 9% hip height reduction in the degus. The kyphotic spine of the ferret compared to the straight, beam-like spine of the ferret in the tunnel resulted in a 34% reduction in peak spine height between experimental conditions. The peak spine height of the degus was reduced by 14% in the tunnel chamber (Table 1).

Vegas, NV, USA) set up in a 'pull' flow-through system (Melanson et al., 2010). All metabolic measurements were recorded in a room maintained at 21.5±0.5°C. Custom-built Plexiglas chambers were used to collect respired gases from the animals. The chamber dimensions for the ferrets were 16.5 cm width×53 cm length; the heights of the chambers were 23 cm and 10.5 cm for unconstrained and constrained trials, respectively. The degu chambers were 16.5 cm width×39 cm length and the heights were 11 cm (unconstrained) and 8.5 cm (constrained). Animals of both species were willing to enter smaller tunnels, but were unable to match treadmill speeds if chamber heights were reduced beyond the abovementioned dimensions. Air was pulled through the chambers via an integrated mass flow generator, which maintains flow rates at values corrected to standard temperature and pressure. Flow rates that were high enough to collect all expired air were determined for each chamber and species, and ranged from 25 to 30 liters min^{-1}. For ferrets, the excurrent air was pulled at a greater rate (45–50 l min^{-1}) from the rear of the chamber so that various stimuli could be used to entice the animals to maintain a forward-facing position relative to the treadmill (Fig. 1). For degus, air was pulled from the anterior-most portion of the chamber. Resting metabolic rates (RMRs) were recorded at least ten times for each individual while sitting quietly in a covered chamber for ~10 min, and the average of the three lowest resting bouts were used for all subsequent calculations.

For locomotion trials, each animal was tested over a range of speeds spanning relatively slow speeds to the fastest possible speed attainable in the height-reduced chamber. Prior to each trial, the animal was allowed to rest for several minutes in the chamber before the treadmill was turned on, then gradually brought up to a targeted speed while metabolic data were monitored. The animal acclimated to the speed for at least 2 min, and metabolic data were recorded at 1 Hz for several minutes or until metabolic recordings reached steady-state (less than 5% variation). Experiments were discontinued if an animal did not match treadmill speed or displayed higher than usual metabolic rates (indicating stress). The fractional compositions of O_2 and CO_2 from the excurrent air were measured from air subsampled at a rate of 200 ml min^{-1}. Water vapor and temperature were continuously measured with high precision and accuracy (accuracy better than 1.0%, resolution 0.001% RH), and calculations of flow and gas concentrations were then corrected for the contribution of water vapor following methods detailed and validated elsewhere (see Lighton, 2008; Melanson et al., 2010 for more detail). Briefly, flow rate, O_2 and CO_2 were adjusted for the dilution effects of water vapor pressure present in the sample as an alternative to

completely desiccating the air sample. These adjusted values were used to calculate metabolic rates, as described below.

Calculations

Data were analyzed in Expedata (Sable Systems, Las Vegas, NV, USA). Metabolic rate (rate of oxygen consumption; $\dot{V}_{O_2}$ in ml O_2 min^{-1}) was calculated using respirometry equations adapted from Withers (1977), and following Melanson et al. (2010):

$$\dot{V}_{O_2} = \mathrm{FR} \cdot [(F_{\mathrm{in},O_2} - F_{\mathrm{ex},O_2}) - F_{\mathrm{in},O_2} \cdot (F_{\mathrm{ex},CO_2} - F_{\mathrm{in},CO_2})]/(1 - F_{\mathrm{in},O_2}), \tag{1}$$

where FR is the excurrent flow rate adjusted for standard temperature and pressure (STP) and water vapor pressure, F_{in,O_2} is the fractional incurrent concentration of oxygen and F_{ex,O_2} is the fractional excurrent concentration of oxygen. Net metabolic rate ($\dot{V}_{O_2,\mathrm{net}}$) was calculated by subtracting an individual animal's RMR from each exercise trial. Net mass-specific metabolic rate was determined as $\dot{V}_{O_2,\mathrm{net}}/M_b$, where M_b is body mass (kg).

The cost of transport (energy cost of moving a unit of mass over a unit of distance; CoT) was calculated by dividing the mass-specific metabolic rate by speed; this is reported in units of J kg^{-1} m^{-1} by using the conversion factor of 20.1 J ml^{-1} O_2 (Taylor et al., 1972). Net cost of transport (CoT$_{\mathrm{net}}$) was obtained by dividing the net (individual RMRs subtracted from total costs) mass-specific metabolic rate by speed.

The predicted CoT$_{\mathrm{net}}$ was calculated following the equation derived for terrestrial locomotion by mammals (Taylor et al., 1970):

$$M_{\mathrm{terr}} = 8.46 M_b^{-0.40}, \tag{2}$$

where M_{terr} corresponds to the net minimum CoT (CoT$_{\mathrm{min}}$) in ml O_2 kg^{-1} km^{-1}, and M_b is body mass in g. For purposes of comparing across multiple studies, the total cost of transport (CoT$_{\mathrm{tot}}$) was also calculated. The minimum CoT$_{\mathrm{tot}}$ was determined for each individual. The ratio for unconstrained and tunnel conditions were measured by taking an individual's overall mean $\dot{V}_{O_2}$ and dividing by its mean RMR.

Energetics variables for each species were compared to those reported in previous studies. The predicted rate of oxygen consumption during terrestrial quadrupedal locomotion was calculated using the following equation from Taylor et al. (1982):

$$\dot{V}_{O_2}/M_b = 0.53 M_b^{-0.32} v + 0.30 M_b^{-0.31}, \tag{3}$$

where $\dot{V}_{O_2}/M_b$ is mass-specific metabolic rate (ml O_2 kg^{-1} s^{-1}), M_b is body mass (kg), and v is velocity (m s^{-1}). Predicted CoT values

Table 2. Individual variation in resting metabolic rates (RMRs) and net cost of transport (CoT$_{\mathrm{net}}$)

Species	Individual no.	RMR (ml O_2 min^{-1})	Unconstrained			Tunnel		Predicted* minimum CoT$_{\mathrm{net}}$
			Velocity (m s^{-1})	CoT$_{\mathrm{net}}$ (J kg^{-1} m^{-1})		Velocity (m s^{-1})	CoT$_{\mathrm{net}}$ (J kg^{-1} m^{-1})	
Ferret	1	12.74±1.40	0.60±0.08	7.92±1.43		0.54±1.58	10.85±1.69	10.66
	2	15.06±1.70	0.64±0.10	3.13±0.58		0.58±1.36	3.96±0.78	11.22
	3	14.47±0.73	0.63±0.08	7.97±1.52		0.58±0.08	9.76±1.11	10.52
	4	14.18±0.79	0.66±0.09	5.04±0.79		0.51±0.10	7.48±1.03	10.32
	Average	14.11±1.19	0.63±0.10	6.14±1.73		0.55±0.09	8.26±2.13	10.69±0.21
Degu	1	4.65±0.26	0.41±0.06	16.00±3.23		0.35±0.03	16.04±2.00	17.65
	2	4.50±0.03	0.35±0.03	15.65±1.69		0.35±0.03	22.26±1.98	18.74
	3‡	4.83±0.10	0.27±0.04	18.80±2.58		0.25±0.03	22.15±2.05	19.25
	Average	4.66±0.13	0.34±0.04	16.78±2.59		0.32±0.03	19.76±2.63	18.40±0.37

*Predicted from equation derived in Taylor et al., 1982.
‡Female degu; all other animals of both species were males.
Means±s.e.m. are shown.

were compared to the average of experimentally obtained values in this study.

Statistical analyses were performed with Systat v. 11.0. Regression equations were constructed using SigmaPlot (Jandel Scientific Software). In order to minimize the effects of inter-individual variation, mixed model ANCOVAs were performed with individual as a random factor and velocity as a covariate.

RESULTS

Ferret kinematics and energetics

In the ferrets, the tunnel challenge resulted in a 23.5% decrease in hip height and a 34% reduction in back height from unconstrained locomotion (Table 1, Fig. 1). Attempts to increase speed ranges were thwarted by the ferrets' inability to move faster than ~0.8 m s^{-1} in the tunnel and their tendency to wander erratically in the unconstrained chamber at the slowest speeds. The overall range of speeds in each condition was approximately four-fold: 0.21 m s^{-1} to 0.83 m s^{-1} in the tunnel; 0.25 m s^{-1} to 1.0 m s^{-1} in the unconstrained condition. Stride frequency increased linearly in both conditions as a function of speed. Although stride frequency was slightly higher in tunnel conditions, the difference was not significant (means±s.e.m.: tunnel, 3.09±0.05 Hz; unconstrained, 2.94±0.06 Hz; $F_{1,3}$=3.23, P=0.07).

While on the treadmill, the ferrets never performed any gaits with an aerial phase, nor did they perform asymmetrical gaits. Limb phase, the proportion of a stride cycle occurring between ipsilateral foot falls, was nearly identical between conditions (tunnel=0.25±0.03; unconstrained=0.2±0.03), and thus the ferrets moved primarily with lateral sequence singlefoot gaits regardless of height constraints. Although similar gait patterns are described elsewhere as 'walks' (e.g. Fish et al., 2001; Williams, 1983), data from previous studies on the same individuals (Horner and Biknevicius, 2010) demonstrated that the ferrets move predominantly in 'grounded runs'; that is, the whole-body external mechanical energy profiles are characteristic of running mechanics despite moving without an aerial phase (Reilly and Biknevicius, 2003; Schmitt et al., 2006). Duty factor was slightly greater in tunnel conditions (tunnel=0.78±0.03; unconstrained=0.74± 0.03), but the difference was not significant ($F_{1,3}$=1.21; P>0.05).

The resting metabolic rate for the ferrets averaged 14.11±1.2 ml O$_2$ min^{-1}. Mass-specific mean values can be found in Table 2. Because there was considerable variation among individuals in mass and RMR, all calculations of net $\dot{V}_{O_2}$ and CoT$_{net}$ reflect individual values of RMR rather than species averages. The relationship for total $\dot{V}_{O_2}$ with speed is illustrated in Fig. 2 and indicates that, at any given speed, metabolic rates in tunnel locomotion were greater than during overground locomotion (mixed model ANCOVA; $F_{1,3}$=46.72; P=0.006). The slopes of the equations describing this relationship both within an individual (Fig. 2A,B) and pooled among individuals (Fig. 2C) were significant (P<0.01). However, the coefficient of determination (R^2) was less for mass-specific comparisons (Fig. 2C).

Similarly, the relationship for $\dot{V}_{O_2,net}$ ($\dot{V}_{O_2}$–RMR) with speed indicated that tunnel locomotion is more expensive in ferrets compared to running overground ($F_{1,3}$=31.75; P=0.011). When mass-specific $\dot{V}_{O_2}$ values were compared to those predicted from the equation in Taylor et al. (1982) (Fig. 2C), both tunnel and unconstrained values were found to be significantly lower than predicted, regardless of speed.

The ratio of $\dot{V}_{O_2}$ to RMR during tunnel locomotion was slightly higher than during overground locomotion, but the difference was not statistically significant (Table 3).

CoT$_{net}$ values for the ferrets were obtained for each trial by dividing the net metabolic rate by the speed of the trial. CoT$_{net}$ decreased curvilinearly with increased speed (Fig. 4A):

$$CoT_{net(unconst)} = 4.35v^{-0.66}, \tag{4}$$

$$CoT_{net(tunnel)} = 4.74v^{-0.54}, \tag{5}$$

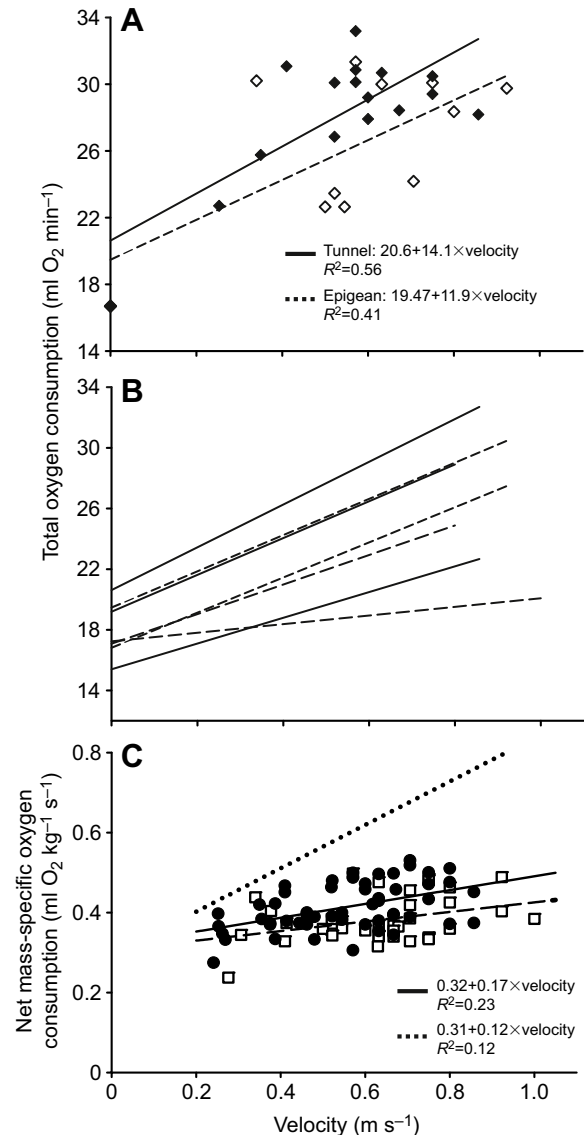where CoT$_{net}$ is in J kg^{-1} m^{-1} and v is velocity in m s^{-1}.

Fig. 2. Regressions of $\dot{V}_{O_2}$ and speed for ferrets running in tunnels and open chambers. Tunnels: black symbols, solid lines; open chambers (epigean): white symbols, long-dashed lines. y-intercepts were derived from averages of measured resting metabolic rates for all individuals; equations for $\dot{V}_{O_2,net}$ are displayed for each condition in the form of intercept + slope × velocity. (A) Relationship between total oxygen consumption and speed in one representative ferret. (B) Regressions of speed versus rate of total oxygen consumption for four male ferrets in tunnels and in open chambers. Masses ranged from 890 g to 1150 g. Regressions for tunnel trials averaged an R^2 of 0.52±0.16; for epigean (open chamber) trials the mean R^2 was 0.40±0.05. (C) Net (total rate minus resting cost) mass specific metabolic rate versus speed pooled among individuals in tunnels (black circles, solid line) and in overground (white squares, long-dashed line). The short-dashed line is the predicted relationship based on the mean mass of the ferrets (from Taylor et al., 1982).
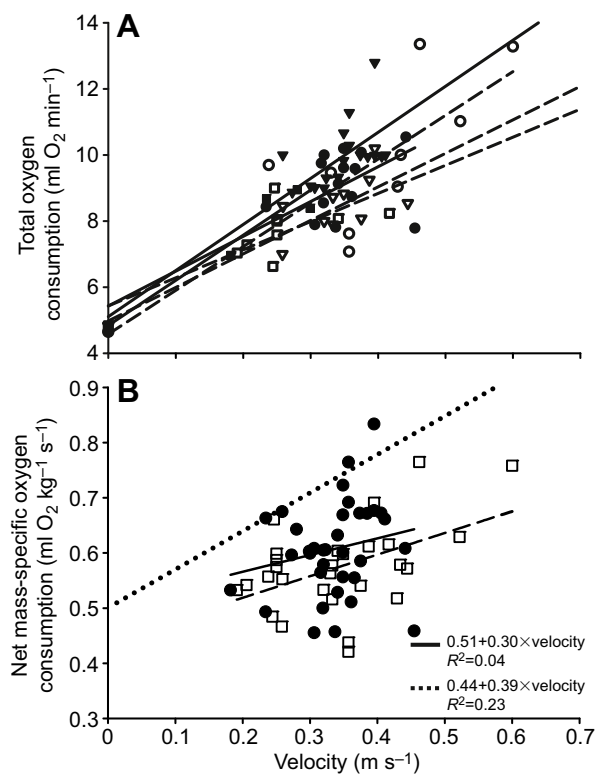
Table 3. Sample sizes and average ratios of $\dot{V}_{O_2}$ to RMR

Species	Unconstrained		Tunnel		$\dot{V}_{O_2}$:RMR tunnel/$\dot{V}_{O_2}$:RMR unconstrained
	No. of trials	$\dot{V}_{O_2}$:RMR	No. of trials	$\dot{V}_{O_2}$:RMR	
Ferrets	39	1.94±0.22	55	2.11±0.26	1.088
Degus	28	1.89±0.21	35	2.05±0.15	1.085

The right-hand column is the difference in metabolic scope between tunnel and unconstrained locomotion.

made between species. As with the ferrets, the overall range of speed in the degus was higher in the unconstrained conditions (0.2–0.6 m s⁻¹) when compared to the tunnel (0.19–0.45 m s⁻¹), despite many attempts made to capture a wider range of speeds.

Degu individual and mean RMR values may be found in Table 2. In the degus, both total (Fig. 3A) and net (Fig. 3B) values of oxygen consumption exhibited similar linear relationships with speed in tunnel and unconstrained conditions. The relationship between degu $\dot{V}_{O_2,net}$ and speed is described by:

$$\dot{V}_{O_2,net(unconstr)} = -0.19 + 12.6v, \tag{6}$$

$$\dot{V}_{O_2,net(tunnel)} = 1.5 + 9.9v. \tag{7}$$

Degu $\dot{V}_{O_2,net}$ was significantly higher in the tunnel than in the unconstrained condition (one-way ANCOVA: tunnel=4.89± 0.18 ml O₂ min⁻¹; unconstrained=4.08±0.20 ml O₂ min⁻¹; $F_{1,60}$=8.94; P=0.004).

When degu mass-specific metabolic rates were compared to predictions based on the Taylor et al. (1982) equation (Fig. 3B), degu values were significantly lower in both conditions.

Although the challenges (in terms of ceiling height) were quite different, the ratio of metabolic rate in tunnels to resting metabolic rate ($\dot{V}_{O_2}$:RMR) was very similar to that of the ferrets (Table 3). Degu CoT_net decreased curvilinearly with speed (Fig. 4B), and is described by the following equations:

$$CoT_{net(unconst)} = 10.85v^{-0.36}, \tag{8}$$

$$CoT_{net(tunnel)} = 12.02v^{-0.42}. \tag{9}$$

Fig. 3. Regressions of $\dot{V}_{O_2}$ and speed for degus running in tunnels and open chambers. Tunnels: black symbols, solid lines; open chambers: white symbols, long-dashed lines. *y*-intercepts were derived from averages of measured resting metabolic rates for each individual; equations for $\dot{V}_{O_2,net}$ are displayed for each condition in the form of intercept + slope × velocity. (A) Regressions of speed versus rate of total oxygen consumption for two male and one female (square symbols) degu in tunnels and in open chambers. Masses ranged from 220 g to 290 g. Regressions for tunnel trials averaged R^2 values of 0.73±0.19; for epigean trials the mean R^2 was 0.69±0.07. (B) Net mass-specific metabolic rate versus speed pooled among individuals in tunnels (black circles, solid line) and open chambers (white squares, long-dashed line). The short-dashed line is the predicted relationship (from Taylor et al., 1982).

Degu kinematics and energetics

Unlike the ferrets, the degus would not readily perform on the treadmill at greater than a 20% reduction in hip height. The minimum heights for which all animals within each species could move on the treadmill resulted in 8.7% reduction in hip height and ~13% reduction in back height for the degu (Table 1, Fig. 1). Each species exhibited different threshold heights, and thus no statistical comparisons were

DISCUSSION
Semi-fossorial morphology: musteline and 'generalized'
A large diversity of morphologies is found among semi-fossorial mammals, which may be in part explained by the duality of the selective forces acting upon them. Semi-fossorial mammals must be able to dig and/or move in tunnels yet also be able to navigate
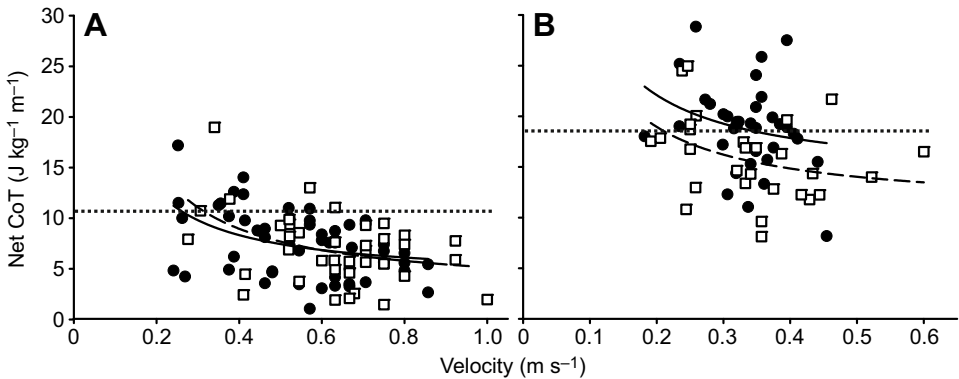

Fig. 4. Net cost of transport (CoT) for ferrets and degus in tunnels and in open chambers. (A) Ferrets. (B) Degus. Tunnels: black circles, solid lines; open chambers: white squares, long-dashed lines. The short-dashed lines represent the predicted net CoT based on the average mass of the animals used in the study.
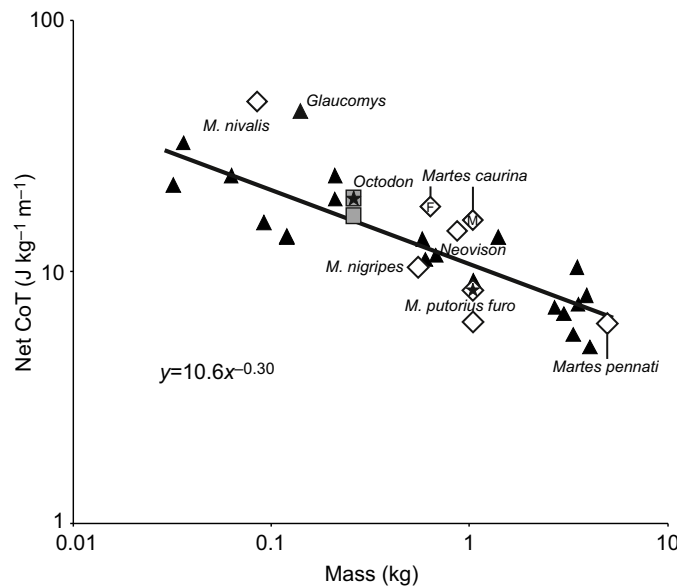
Fig. 5. Net cost of transport (CoT) of terrestrial locomotion plotted against body mass in a range of small to medium mammals under 10 kg in size. Some mustelids (labeled by species, diamond markers) and the highly specialized flying squirrel (labeled *Glaucomys*) exhibit a higher CoT than predicted by size, even in this smaller cohort. Degus (gray squares; labeled *Octodon*) and ferrets from this study are included in the graph, but were not used in the calculation of the allometric equation; tunnel trials were averaged among individuals and indicated with a star. CoT values from mustelids (diamonds) and other <10 kg mammalian taxa (triangles) were obtained from: Chappell et al. (2013), Fish and Baudinette (1999), Fish et al. (2001), Flaherty et al. (2010), Flaherty et al. (2014),Taylor et al. (1982), Williams (1983); F and M indicate female and male individuals.

overground, unconstrained environments. Some have shorter limbs relative to terrestrial generalists, perhaps as an adaptation for accommodating tunnels and burrows (Nevo, 1979). However, shorter-limbed animals must take a greater number of steps to traverse a given distance, thus increasing the energetic cost of transport (Heglund and Taylor, 1988; Kram and Taylor, 1990; Taylor, 1985). A study of the short-limbed, semi-aquatic mink (Carnivora: Mustelidae, *Neovison vison*) found that the energetic cost of terrestrial transport was nearly 25% higher than predicted for a similarly sized terrestrial generalist with longer limbs (Williams, 1983). The ferrets in the present study, however, exhibit lower than predicted net energetic costs of terrestrial transport (Fig. 5).

The ferrets did incur higher energetic costs when moving in tunnels compared to the overground, unconstrained condition. The elevated cost was not due to differences in speed, stride frequency or gait, as tunnel and unconstrained trials were similar with regard to all of these factors; indeed, a previous study on the same individuals (Horner and Biknevicius, 2010) found that not only were velocities and symmetrical gaits similar between tunnel and overground conditions, but no differences were found in center-of-mass mechanics; i.e. ferrets in both conditions most commonly moved in grounded runs (spring mass mechanics without an aerial phase).

The lack of biodynamic differences between overground and tunnel conditions is especially surprising given the substantial change in posture that occurs when ferrets enter a tunnel. Ferrets moving overground retain a stiffly arched back, such that the distance between pectoral and pelvic girdles (the functional trunk length) is close to the proportions of other similarly sized (non-musteline) mammals (Moritz et al., 2007). Upon entering the

confined heights of tunnels, the animals must extend their spines to lower their dorsal profile, thereby increasing the functional trunk length by up to 30% (Horner and Biknevicius, 2010; Moritz et al., 2007). Additionally, considerable flexion of the limbs (~25% reduction in hip height in both studies) occurs in order to accommodate the body into the tunnel. The combination of spinal extension and limb flexion results in height reductions of 34–40% in the overall dorsal profile of the ferrets (Horner and Biknevicius, 2010; present study).

These postural shifts likely account for the higher energetic costs of tunnel locomotion in ferrets, as both spine extension and limb flexion are accomplished by internal work (i.e. muscular effort). All terrestrial mammals must engage muscles of limb extension to counteract the tendency for limbs to collapse into flexion during stance phase (Biewener, 1989). One evolutionary strategy to maintain posture more efficiently and reduce musculoskeletal stresses is the alignment of the limb joints and segments with the ground reaction force (GRF) vector. This relationship of limb posture to GRF is the limb effective mechanical advantage (EMA), and EMA tends to increase as a function of body size in mammals due to more erect posture (Biewener, 1989). Because of poor limb EMA, the extensor muscles in small, crouched mammals must be activated nearly throughout stance phase to maintain posture (Gillis and Biewener, 2001), whereas larger mammals rely more on tendinous elements and erect limbs to prevent limb collapse during stance (Roberts, 1998). When a crouched mammal moves with an even greater degree of flexion – as in the increasingly crouched limb posture exhibited by ferrets in tunnels – a further reduction of EMA occurs and thus additional compensatory muscle forces are required.

The shift in spine posture from arched to extended is also expected to increase the energetic cost of tunnel locomotion. Spinal extension effectively elongates functional trunk length, which increases the rotational forces acting upon the pectoral and pelvic girdles (Fischer, 1994; Moritz et al., 2007). To maintain spinal posture, these rotational forces must be counteracted by epaxial muscles such as the sacrospinalis muscles. There is evidence that ferrets have physiological adaptations to mitigate at least some of this energetic cost (Moritz et al., 2007): out of a sample of small mammal species, the epaxial muscles of ferrets (including sacrospinalis and iliopsoas muscles) were found to possess unusually high proportions of oxidative, slow-twitch fibers, similar to the composition of fiber types found in other high-endurance postural muscles such as soleus. Other small mammals commonly mobilize the lumbar portion of the spine during asymmetrical gaits, and have correspondingly high proportions of glycolytic fibers. The fiber-type data suggests that the epaxial muscles of ferrets are utilized to stabilize, rather than mobilize, the trunk.

Unlike other fossorial hystricomorphs, the semi-fossorial degu does not have any obvious anatomical adaptations for digging despite building colonial burrows (Ebensperger and Bozinovic, 2000a). The degu has a generalized small mammal body plan in that their trunks are relatively short and limbs crouched. Like all small mammals, degus have a kyphotic spine (Gambaryan, 1974) but with a much reduced dorsal height compared to the ferrets. As with the ferrets, the tunnel condition represented a significant challenge for the degus, demonstrated by higher energetic costs when moving in the height-reduced chambers. These results are somewhat surprising given the different morphologies of the rodents compared to ferrets. Because of the reduced trunk length, the degus were not required to extend their spines to the same degree as the ferrets, and thus may be expected to more easily enter tunnels. However, degus exhibited

very different minimum threshold height tolerances (relative to standing back height) than the ferrets. The degus could only consistently perform with a 13% reduction in back height, achieved by a near-equal effort of limb flexion and spinal extension (similar to the ferrets). Given the relatively small changes in hip height, this reduction must have been accomplished primarily by spinal extension. To truly delineate the morphological influences of tunnel locomotor efficiency, data from a broader taxonomic and phylogenetic sample are necessary.

Ferrets and degus displayed modest increases in the ratio of exercising metabolic rates to RMRs between tunnel and unconstrained locomotion (∼8%; Table 3), despite the very different postural challenges posed by the tunnel condition for each species. These ratios (∼2× RMR for tunnel locomotion; Table 3) are relatively small when compared to such rigorous activities as burrowing (which can be up to 7× RMR; Ebensperger and Bozinovic, 2000b; Luna et al., 2002) or $\dot{V}_{O_2,max}$ which has been reported as 9× RMR in both weasels (Chappell et al., 2013) and mink (Williams, 1983).

Cost of transport

Cost of terrestrial transport values in specialized animals are usually higher than for generalists, and the same is often true of semi-specialized animals. In analyses of CoT values among semi-aquatic mammals, terrestrial CoT was 25% (mink; Williams, 1983), 40% (Williams et al., 2002), and even 70% (water rat; Fish and Baudinette, 1999) higher than predicted values. Although semi-fossorial mammals may not have the same suite of morphological adaptations that semi-aquatic mammals possess (e.g. webbed feet), some compromise in terrestrial performance might be expected. Some researchers have observed higher than expected CoT values in mustelids, such as the least weasel (*Mustela nivalis*, Fig. 5; Chappell et al., 2013) and the Pacific marten (*Martes caurina*, Fig. 5; Flaherty et al., 2014), crediting the distinctive morphology of the mustelid form as responsible. Yet, among several other long-bodied, short-limbed mustelids, CoT for overground locomotion was often similar to predicted values (*M. nigripes* and *Martes pennati*, Fig. 5; Taylor et al., 1982) or even lower than predicted (*M. putorius furo*; this study). Thus, the hypothesis that a mustelid body form – elongate, relatively shorter limbs – would be less efficient during overground locomotion is not well-supported.

There are several possible explanations for these unexpected results, including the role of RMRs and how CoT is determined. RMR data are representative of an animal's metabolism during wakeful rest, and constitute a significant portion of total daily energetic costs. Variation in RMR between individuals was considerable, and this variation may have obscured the effects of the experimental treatment somewhat. As has been suggested by other authors (Lighton, 2008; Williams et al., 2002), this study advocates the approach of obtaining each individual animal's RMR to calculate net values of $\dot{V}_{O_2}$ and CoT. Calculating the data in this manner also permits comparison of the ferret data to other locomotor studies (e.g. Chappell et al., 2013; Flaherty et al., 2014). An alternative explanation, but not mutually exclusive to RMR, may lie with differences in the relationship between CoT and speed between large and small animals. Mass-specific CoT, the dimensionless value that represents the energetic cost to move a unit of mass over a unit distance, is a convenient measure of locomotor efficiency that may be used to compare morphologically and phylogenetically disparate taxa; when coupled with mass, over 94% of variation in metabolic rates in birds and mammals may be explained by behavioral and ecological variation, with phylogeny contributing less than 5%

(McNab, 2015). Within a gait, a linear relationship between metabolic rate and speed exists in many mammals, including humans and horses (Wickler et al., 2001); thus, net CoT can simply be determined as the slope of this line. Among smaller (<5–10 kg) animals, metabolic rate versus speed is more variable, and CoT usually decreases curvilinearly with speed when an animal's natural range of speeds are used, regardless of gait choice (Bethge et al., 2001; Fish and Baudinette, 1999; Fish et al., 2001; Flaherty et al., 2010; Fournier and Weber, 1994; Williams, 1983; this study). In our study, the subjects did not appear to utilize speeds that minimized their energetic costs. Our data agree with those from other studies that indicate that small mammals do not move efficiently, as a rule; they exhibit poor EMA, use relatively high stride frequencies and tend to move in costly, intermittent bouts (Biewener, 1989; Fischer, 1994; Gleeson and Hancock, 2001; Hodson-Tole and Wakeling, 2010). Rather than optimize efficiency, small mammals seem to optimize maneuverability and acceleration (Fischer, 1994).

Further caveats of laboratory-based performance studies of small mammals are the range of speeds attained in laboratory settings versus natural conditions, as well as individual variation. When CoT values are captured from only the fastest end of the animal's repertoire, results are likely to be skewed. In this study, ranges of preferred speeds in the ferrets were measured in an earlier study (on a trackway, rather than treadmill) on the same individuals, and thus no *a priori* assumptions of 'optimal' speed were made. It is unlikely that most small mammals, which tend to move intermittently with many accelerations and decelerations, target a single optimal speed or minimum CoT. Additionally, animals that are not calm or physically fit can inflate metabolic rate data substantially.

Conclusions

Although we cannot compare results from ferrets directly to degus (e.g. Garland and Adolph, 1994), our within-species results support the conclusions that tunnel locomotion is more energetically costly compared to terrestrial locomotion, and postural changes likely drive this cost. Degus and ferrets did not appear to demonstrate a 'cost of semi-specialization' as measured by percent difference from allometric predictions. For ferrets, though tunnel locomotion does come with an increased metabolic cost, their transport costs in both conditions were surprisingly less than that of similarly sized terrestrial mammals. Although we did not explore the mechanics of the efficiency of ferret locomotion here, previous studies have observed unique histochemical (Moritz et al., 2007) and postural (Horner and Biknevicius, 2010) properties that may be adaptive for tunnel and overground locomotion. Due to the ubiquity of tunnel utilization among mammals, further studies may reveal a more diverse suite of adaptations in other semi-fossorial animals.

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