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The net energetic cost of terrestrial locomotion by the snake *Coluber constrictor*, moving by lateral undulation, is equivalent to the net energetic cost of running by limbed animals (arthropods, lizards, birds, and mammals) of similar size. In contrast to lateral undulation and limbed locomotion, concertina locomotion by *Coluber* is more energetically expensive. The findings do not support the widely held notion that the energetic cost of terrestrial locomotion by limbless animals is less than that of limbed animals.

SPECIES WITH REDUCED LIMBS OR NO LIMITS AND elongate bodies have evolved independently from limbed antecedents in several groups of vertebrates: salamanders, cecilians, amphibiaesians, lizards, and snakes (1). An important factor proposed to explain the evolution of limblessness is its presumptively low energetic cost, such that energetic expenditure during locomotion by limbless animals is expected to be less than that of limbed animals of similar size (1, 2). Biomechanical arguments advanced in support of the low energetic cost of limbless locomotion include no costs associated with vertical displacement of the center of gravity (1, 3, 4), no costs to accelerate or decelerate limbs (3), and low cost for support of the body (1). A preliminary study, published only as an abstract, reported that the energetic cost of locomotion of the garter snake (*Thamnophis sirtalis*) was only 30% of that predicted for a quadrupedal lizard of similar size (5). Although that study was preliminary, it has been widely cited in review articles (1-3, 6, 7) and textbooks (4, 8) in support of mechanical arguments for the low cost of limbless locomotion. We sought to test the generality of these conclusions by examining the energetic cost of locomotion in a snake, the black racer (*Coluber constrictor*).

A snake may utilize a variety of locomotor modes, depending on both speed and surface encountered (4, 9-11). Lateral undulation and concertina locomotion are two common modes that use lateral vertebral movements to generate propulsive forces. During lateral undulation on the ground, snakes move along an approximately sinusoidal trajectory. Bends in the body contact with the substrate and push posteriorly on projections from the ground, propelling the body forward. All parts of the body move simultaneously with the same overall speed, while forward and lateral components of velocity change as a result of the sinusoidal trajectory (10, 11). Snakes moving with lateral undulation experience only sliding contact with the ground (4, 12). In narrow passageways such as tunnels, snakes often perform concertina locomotion exclusively (4). Snakes performing concertina locomotion stop periodically, and certain parts of the body are moved forward while others maintain static contact with the ground. In passageways, snakes alternately press themselves against the sides by forming a series of bends and then extend themselves forward from the region of static contact (4, 13). In comparison to lateral undulation, concertina locomotion involves higher momentum changes (4), resistance due to static (as well as sliding) friction (4), and usually slower forward speed (11) and, therefore, probably entails higher energetic costs.

Although these considerations logically suggest differential costs of the two locomotor modes, only if we directly determine the metabolic rates of moving animals can these be verified and compared to anticipated values for limbed animals. In the current study, we measured energy expenditure as the rate of oxygen consumption of snakes at rest (VO₂rest), in the moments just before locomotory exercise (VO₂pre-ex), and during locomotion at several speeds (0.2 to 1.0 km hour⁻¹ for lateral undulation, 0.06 to 0.14 km hour⁻¹ for concertina locomotion) on motorized treadmills (14, 15). Endurance, measured as time sustained on tread, as a function of speed and locomotor mode was also determined (16). Videotapes were used to verify locomotor mode and to correlate frequency of movement with energy expenditure. By dividing oxygen consumption by frequency of movement, we estimated energetic costs of single cycles of lateral undulatory and concertina movement.

The metabolic response of VO₂ to speed in *Coluber constrictor* [mass = 102.8 ± 6.1 (SE) g, n = 7] locomoting by lateral undulation is similar to that observed in many terrestrial vertebrates with limbs (17): VO₂ increases as a linear function of speed (18) throughout the range of sustainable speeds (0.2 to 0.5 km hour⁻¹), above which VO₂ is constant and endurance decreases (speeds greater than 0.5 km hour⁻¹) (1 Fig. 1, A and B). Oxygen consumption also increased as a
linear function of speed during concertina locomotion by *C. constritor* (19) but with a greater slope than that of lateral undulation (t = 3.67, df = 28, P < 0.001, Fig. 1A). The point of intersection between the increasing and constant phases of the metabolic response plot defines the maximal rate of oxygen consumption (VO₂ max) and the lowest speed at which VO₂ max is achieved, often termed the maximum aerobic speed (MAS) (20). Cohler *constritor* performing lateral undulation achieves VO₂ max = 0.83 ml of O₂ per gram per hour ± 0.06 (n = 5) at MAS = 0.5 km hour⁻¹ (Fig. 1A). This VO₂ max is nine times the resting rate and is similar to the maximum rate previously reported for this species (21). The MAS is one-tenth of the maximum burst speed (22) of these animals (mean maximum burst speed = 5.5 ± 0.4 km hour⁻¹). Endurance decreased too precipitously during concertina locomotion (Fig. 1B) to permit us to determine VO₂ max and MAS for this locomotor mode.

Rates of oxygen consumption extrapolated to zero speed (the y intercept) are often elevated above resting rates, and earlier investigators have interpreted this increment as the energetic cost of postural support (20). For *C. constritor* performing lateral undulation, the y intercept is elevated above zero (t = 2.61, df = 21, P < 0.02) but is indistinguishable from the resting (t = 1.39, df = 21, P > 0.10) or pre-exercise (t = 0.33, df = 21, P > 0.50) rates of oxygen consumption (Fig. 1A). The y intercept for the metabolic response during concertina locomotion is indistinguishable from zero (t = 0.89, df = 7, P > 0.40, VO₂ rest (t = 1.50, df = 7, P > 0.10), and VO₂ pre-ex (t = 2.36, df = 7, P > 0.50). The y intercepts for the metabolic responses during concertina and lateral undulation are also indistinguishable from each other (t = 1.84, df = 28, P > 0.05).

Snakes increase speed by increasing the number of cycles of movement per unit time (Fig. 2A). The frequency of lateral undulation continues to increase beyond the range of sustainable speed. Thus, undulation at nonsustainable speeds is probably supported by both aerobic metabolism and increasing contributions of anaerobic metabolism. The energetic cost of a single cycle of lateral undulation does not change within the range of aerobically sustainable speeds [F(3, 9) = 2.57, P = 0.12] (Fig. 2B) but does vary significantly among individual snakes [F(3, 26) = 33.9, P < 0.001] (23).

Concertina locomotion has an increased energetic cost compared to that of lateral undulation. The oxygen consumption of animals performing concertina locomotion exceeds VO₂ predicted for snakes performing lateral undulation at similar speeds (Fig. 1A). Furthermore, endurance during concertina locomotion is much less than for lateral undulation at similar speeds (Fig. 1B). The elevated energetic cost of concertina locomotion is attributable to two factors: (i) snakes performing concertina locomotion require more cycles of movement to sustain the same speed than they do during lateral undulation [two-tailed paired *t* test comparing the rate (in cycles per minute) of animals locomoting at 0.2 km hour⁻¹, n = 5, t = 8.37, P = 0.001, Fig. 2A], and (ii) the energetic cost of a single cycle of concertina locomotion is greater than that of a single cycle of lateral undulation [Mann-Whitney *U* test, *U*(9, 28) = 179, P < 0.001, Fig. 2B].

The energetic cost of locomotion by *C. constritor* is expressed by the slope of the line relating VO₂ to speed within the range of aerobically sustainable speeds (17) (Fig. 1A). This slope, often termed the net cost of transport (NCT), indicates the amount of energy required to move a unit mass of animal a given distance (20) and is frequently used for comparisons among taxa and locomotor modes (24, 25). The NCT of

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**Fig. 1.** (A) Steady-state rate of oxygen consumption (VO₂) as a function of speed for seven individuals performing lateral undulation (C) and three individuals performing concertina locomotion (CS). Mean speed is reported, because animals moving by concertina locomotion periodically stop. The mean resting rate of oxygen consumption is indicated by the x at zero speed; pre-exercise rates of oxygen consumption are indicated by circles at zero speed. The net cost of transport for lateral undulation is represented by the slope of the line in the increasing region of the plot (0.2 km hour⁻¹ ≤ speed ≤ 0.5 km hour⁻¹). The least-squares estimate of the line for this region is VO₂ = 1.153 ±0.0205 SE × speed + 0.222 (±0.085 SE), n = 23, P = 0.0001 (curved dashed lines represent ±95% confidence limits of predicted values of VO₂). An alternative method is to calculate separately a regression for each snake and then determine the mean slope and intercept of these individual regressions. This method indicated a relation similar to that of the model 1 regression: VO₂ = 0.916 ±0.179 SE × speed + 0.285 (±0.069 SE) (n = 6). Oxygen consumption is not related to speed at ≥0.5 km hour⁻¹. The regression equation for this region is VO₂ = -0.069 × speed + 0.845, P = 0.84. The equation relating VO₂ to speed during concertina locomotion is VO₂ = 8.494 ±1.679 SE = 0.151 ±0.170 SE, n = 9, P = 0.002. (B) Endurance (time to exhaustion) as a function of speed. All snakes performing lateral undulation at 0.4 km hour⁻¹ sustained locomotion for 120 min; the trials were stopped at that point.
lateral undulatory locomotion for C. constrictor (1.15 ± 0.21) is virtually equivalent to that predicted for a limbed lizard (26) of similar mass (predicted NCT = 1.14, Fig. 3). In fact, the NCT of C. constrictor is similar to that predicted for terrestrial locomotion of birds, mammals, and arthropods of similar mass (25). Furthermore, the NCT of C. constrictor performing concertina locomotion (8.49 ± 1.68) is seven times the NCT for C. constrictor lateral undulation and substantially greater than that predicted for limbed animals of similar mass (Fig. 3). In contrast to previous observations (5) and widely held opinions concerning the energetic cost of limbless locomotion (1-4, 6-8), lateral undulatory and concertina locomotion by C. constrictor are not more economical than walking or running by limbed animals. Because the data for garter snakes (Thamnophis sirtalis) were published only as an abstract (5), it is difficult to assess this discrepancy. For our study, we verified locomotor mode using videotape; and we used a snake, C. constrictor, that is known to have a relatively high capacity for aerobic metabolism (21).

Thus, we are certain that the data we used to calculate NCT involved only aerobically sustainable speeds. In the earlier study (5), the locomotor mode was not identified, although lateral undulation seems probable, and the snakes in that study were tested at speeds (up to 0.9 km hour−1) that may have elicited extensive anaerobiosis and, therefore, underestimated NCT.

Why is terrestrial limbless locomotion not energetically less expensive than limbless locomotion despite plausible biomechanical arguments for its low cost? Equally plausible arguments suggest that certain energetically costly features used during limbless locomotion may neutralize proposed energetic benefits associated with limblessness. For example, limbless animals probably encounter greater external frictional resistive forces than limbed animals (3). Lateral accelerations of the body during limbless movement should also add to the energetic cost. Finally, limbless locomotion is not necessarily without energetic cost for body support, either from muscular activity to maintain rigidity of the ribs or to elevate the head and anterior regions of the body above the ground as the animal moves.

In addition to multiple independent origins of limblessness in amphibians and reptiles, many fossorial lizards and salamanders are characterized by elongate bodies and small but fully functional limbs (1). One explanation for the presence of small limbs in such taxa is that they represent a transitional stage toward the evolution of limblessness (27). In this view, limbs are seen as encumbrances during locomotion through narrow tunnels or crevices. Our results concerning the high cost of concertina locomotion suggest an alternative hypothesis. Many limbless lower vertebrates must switch to energetically costly concertina locomotion within tunnels. In contrast, small but fully functional limbs enable animals to perform limbed locomotion in narrow tunnels, which may also convey an energetic benefit that favors their evolutionary retention.

REFERENCES AND NOTES

14. The resting rate of oxygen consumption was measured for snakes maintained in darkened isolated, cylindrical plastic metabolic chambers. Snakes were placed in the chambers and left undisturbed for 48 hours at 30°C. [All measurements were conducted at 30°C, a typical field active body temperature for C. constrictor; H. S. Fitch, Univ. Karn. Publ. Mus. Nat. Hist. 18, 381 (1963); H. F. Hirth and A. C. King, J. Herpetol. 16, 101 (1969); L. J. Vitt, Capea 1971, 265 (1971).] Fresh air was ventilated through the chambers at a constant flow rate. At 1900 hours on the day of the measurements, an initial 20-ml gas sample was drawn for oxygen analysis with a gas tight glass syringe. The chamber was sealed and left undisturbed in the darkened temperature cabinet for 3 hours. A second 20-ml gas sample was then withdrawn, and the chamber was ventilated with a constant flow of fresh air. We determined the fraction of oxygen content of the samples by injecting the sample at a constant flow rate into an Anetek S3A oxygen analyzer. Gas samples were injected through columns of water (Driette) and CO2 (Ascarite) absorbers, and before entering sensors, sample volumes were corrected to standard temperature, pressure, and density. The rate of oxygen consumption was determined according to the method of D. Vleck [J. Appl. Physiol. 62, 2103 (1987)].
15. We measured rates of oxygen consumption during locomotor exercise at 30°C over a range of speeds on motorized treadmills during undulation or concertina locomotion. A snake at a body temperature of 30°C was fitted with a lightweight, clear plastic mask and which was secured to the head with a small rubber spacer inserted between the dorsal surface of the head and the mask. Room air was drawn through the mask at a constant flow rate of 200 to 300 ml min−1 by a length of flexible plastic tubing attached to the end of the mask. The expired air stream passed from the mask through absorbent cloths to incineration and was directed through a metered air pump. We determined the oxygen consumption by comparing the fractional oxygen content of room air to that of the expired cloths measured using a carbon dioxide analyser...

Fig. 3. Net cost of transport (NCT) as a function of body mass plotted on a log-log scale. Data for limbed lizards were compiled by John-Alder et al. (26). The solid line indicates the regression line calculated for the lizard data (curved dashed lines indicate ±95% confidence limits for the predicted values of NCT). Vertical bars on the lateral undulation and concertina NCTs for Coluber indicate ±1 SE. The garter snake (Thamnophis) datum is from Chodrow and Taylor (5).

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A two-fold (C2) symmetric inhibitor of the protease of human immunodeficiency virus type-1 (HIV-1) has been designed on the basis of the three-dimensional symmetry of the enzyme active site. The symmetric molecule inhibited both protease activity and acute HIV-1 infection in vitro, was at least 10,000-fold more potent against HIV-1 protease than against related enzymes, and appeared to be stable to degradative enzymes. The 2.8 Å angstrom crystal structure of the inhibitor-enzyme complex demonstrated that the inhibitor binds to the enzyme in a highly symmetric fashion.

**HUMAN IMMUNODEFICIENCY VIRUS (HIV-1) CASCADE**

The activity of the protease is essential for the proper assembly and maturation of the viral capsid and the release of infectious virions for HIV-1 (5). The viral protease is encoded by the gag gene, which is divided between the gag and Gag genes. The gag gene encodes the viral capsid and matrix proteins, while the pol gene encodes the reverse transcriptase, integrase, and protease enzymes.

The gag gene codes for the structural proteins of the virion, including the capsid (CA) protein, which is responsible for the formation of the viral capsid. The pol gene codes for the enzymes involved in reverse transcription, integration, and packaging. The protease enzyme (PR) is responsible for the processing of the gag and pol genes, generating the mature viral proteins.

**Design, Activity, and 2.8 Å Crystal Structure of a C2 Symmetric Inhibitor Complexed to HIV-1 Protease**

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**Abstract:** This study reports the design and characterization of a symmetric inhibitor of HIV-1 protease that exhibits high potency and selectivity. The inhibitor is designed to bind to the enzyme in a symmetrical manner, which provides a basis for the development of more effective antiretroviral therapies.

**Keywords:** HIV-1, protease, symmetric inhibitor, crystal structure, viral assembly.