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Energetics of Caterpillar Locomotion: Biomechanical Constraints of a Hydraulic Skeleton

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Power input increased linearly with speed and was closely associated with changes in cycle frequency. Minimum cost of transport of gypsy moth caterpillars was 4.5 times as high as predicted for vertebrates and arthropods with jointed framework skeletons. Reduced locomotor economy was associated with stride length only one-third or less than that for animals with solid skeletons.

FROM THE NOW CLASSIC WORK OF Taylor, Schmidt-Neilsen, and Rabb (*1*), investigations on the cost of terrestrial locomotion have been conducted on birds (*2*), reptiles (*3*), and amphibians (*4*), as well as adult insects (*5*) and other arthropods (*6*). Remarkably, aerobic metabolism during continuous submaximal performance is essentially a linear function of speed in all these groups, and the minimum cost of transport (M_{run}), obtained from the slope of metabolism versus speed relationships, is surprisingly insensitive to differences in morphology, mechanics, or phylogenetic history (*7, 8*). All of the animals in the studies outlined above possess muscles attached to solid skeletal elements, and muscle contraction against the skeleton produces limb movement. Caterpillars depart markedly from such organization. Their muscles surround a continuous, fluid (or hydraulic) skeleton. Muscle contraction against an incompressible fluid causes movements by elongation of the body. I report that the soft body of caterpillars reduces their range of speeds and the range of stride lengths possible yielding energetic costs of locomotion, which are about four to five times as great as for arthropods and vertebrates that use solid skeletons.

Oxygen consumption of gypsy moth caterpillars (*Lymantria dispar*) walking on a motor-driven treadmill was continuously monitored with an S-3A oxygen analyzer attached to an analog to digital channel of a microcomputer (BBC Acorn Model B). Airflow through the chamber was approximately 130 ml/min. Before entry into the sensor, the air passed through a desiccant to remove water vapor. Performance stabilized within 3 to 5 min, and data were only taken for such performances. Oxygen consumption (standard temperature and pressure, dry) was calculated by standard open flow respirometry equations (*9*).

Movements of the caterpillars during locomotion were recorded by placing a video camera (with macro lens) besides the treadmill, allowing continuous recording of side view (*10*). Body wave production (stride frequency) was determined by counting the movements of the terminal prolegs during specified time intervals. Stride length was calculated by dividing the speed by the frequency of body wave production. Movement of prolegs was obtained by videoanalysis from below as caterpillars walked across a perspex sheet.

Terrestrial locomotion by caterpillars occurs as a result of a progressive series of body waves proceeding from posterior to anterior. Body movement occurs only in a horizontal and a vertical plane. The terminal prolegs move forward and anchor the body. Once these prolegs are set, the body wave proceeds forward and muscles from each body segment contract serially. The movement of different body regions is confined to distinct intervals of the cycle. Once a particular proleg pair has moved and been "planted," there is no further movement by that proleg or body segment until the next cycle (Fig. 1). The head, which moves as a result of hydraulic pressure sweeping forward, is moving almost continuously rather than cyclically (Fig. 1). Only a single wave occurs in the body at any one time.

As speed increases, both stride frequency

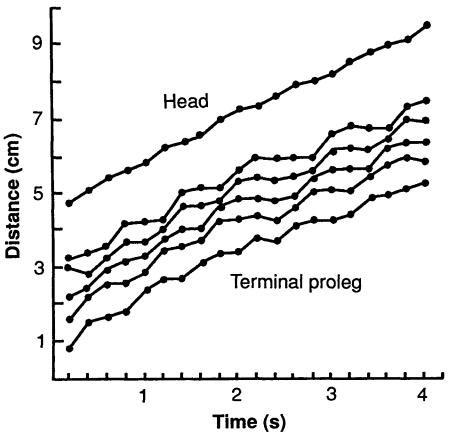

Fig. 1. Movement of the five proleg pairs and the head during several locomotor cycles. As movement is bilaterally symmetrical only the right prolegs are shown.

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and stride length increase significantly (Fig. 2, A and B); the former is more tightly coupled to changing speed. Frequency varied by a factor of four over the speed range whereas stride length varied by about 40%. Oxygen consumption (VO_2) of individual caterpillars increased directly in response to speed (Fig. 2C), varying about threefold over the range of speeds tested. The minimum cost of transport (M_{run} = the slope of VO_2 versus speed) (Fig. 2C) is 22.63 ± 9.35 ml of O_2 per gram of body mass per kilometer traveled (mean mass, 0.85, n = 9). M_{run} for caterpillars differs from the predicted value (5.02) by a factor of 4.5, the difference being outside the 95% confidence limit for the equation (*7, 8*). Extrapolating the individual regression lines for six of the eight individuals to zero speed (Fig. 2C) yielded a metabolic rate of 0.464 ml of O_2 per gram of body mass per hour, only 7% greater than predicted for resting caterpillars of this species at similar body mass and body temperature (*11*). However, extrapolation of the data for two individuals yielded y-intercepts of less than zero, suggesting that there may be a curvilinear relation at low speeds. These data indicate that a hydraulic mechanism for terrestrial locomotion is considerably more expensive than for animals using muscles attached to a solid skeleton.

The major change in power input with speed is associated with changes in locomotor cycle frequency (Fig. 2, A and C). This pattern is comparable to that exhibited by both vertebrate and invertebrate (*13*) pedestrians. The energy cost per stride increases with increasing speed. Over the range of speeds examined the cost increases by about a factor of three. Presumably, increases in speed are associated not only with increases in muscle contraction frequency but also by increased force production by the muscles at higher speeds as indicated by the significant increases in the stride length (Fig. 2B). At the higher speeds [corresponding to the caterpillars' preferred speed both in the laboratory and in the field (*12*)] the mean cost per step was 3.6 J/kg (body mass), similar to the values obtained for arthropods and vertebrates (*13*).

Caterpillar metabolic rates during locomotion are not extraordinary. The increase over rest level is considerably less than the maximal capability for ectothermic vertebrates (*14*) and ectothermic insects (*15*). Moreover, the range of stride frequencies exhibited by the caterpillars are not exceptional and compare well to frequencies of other ectothermic insects during forced activity. Caterpillars' cost per stride is also typical to that of other pedestrians (*13*). Indeed, the proportion of muscle used by caterpillars for locomotion is probably greater than the fraction used by other insects (*12*), so that the cost per stride per gram of muscle is probably lower than for other insects.

The explanation for their higher costs of transport appears to rest in biomechanics rather than in their energetics or muscle performance. Caterpillars operate over a narrow range of slow speeds and exhibit low increase in total speed for an increase in energy input. In running vertebrates much energy that would otherwise be lost in a stride is temporarily stored in elastic structures and used in initiating the next stride (*16*). Arthropods with solid skeletons appear to employ similar mechanisms (7). In caterpillars, even when parts of the body are moving, most of the prolegs remain in contact with the ground (Fig. 1). Thus, little momentum can be built up and each body segment must be reaccelerated from rest at the beginning of the next body wave. Moreover, constrained by their morphology, caterpillars are capable of producing stride lengths of only 25 to 30% of the predicted values for vertebrates and insects of similar mass with solid skeletons, which explains much of the reduction in economy of locomotion. Caterpillar organization precludes the possibility of high-speed locomotion comparable to animals with solid skeletons due to the morphological organization and pattern of movement.

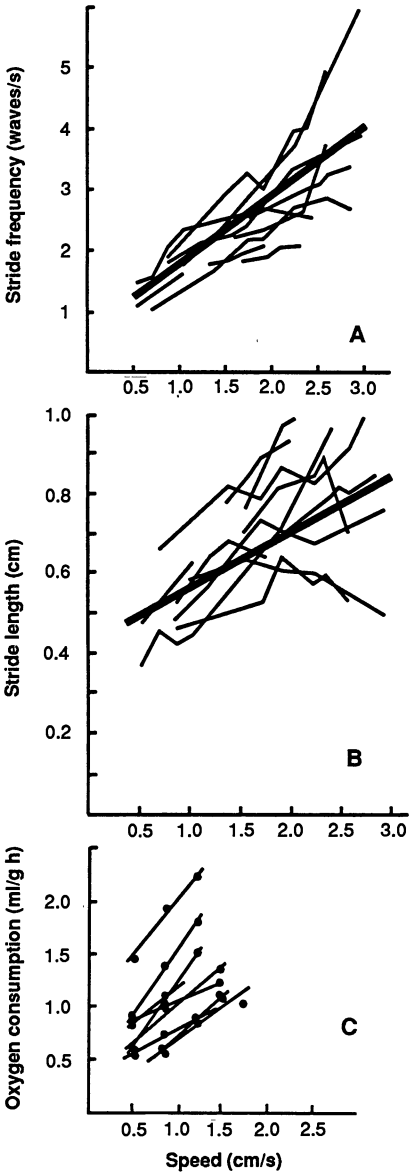

Fig. 2. Performance of caterpillars at controlled speeds on a motor-driven treadmill. (**A**) Stride frequency as a function of crawling speed. Each line represents a separate individual. Dark solid line is linear regression through all data. (**B**) Stride length in relation to crawling speed. (**C**) The relation of steady-state oxygen consumption to crawling speed. Each line represents the linear regression through data for different individuals. Caterpillars could not maintain their performance for long periods at the higher speeds; therefore, cost per contraction at the highest speeds is calculated assuming a linear increase of oxygen consumption with speed.

A comparison of the locomotory performance of caterpillars with that of terrestrial vertebrate ectotherms is of considerable comparative interest not only because of their widely divergent phylogenetic histories but also because evolution in these groups appears to have resulted in highly different responses to similar selective pressures. Among vertebrate ectotherms, there is a strong reliance on burst activity and sprint capacity presumably in response to strong selection pressures associated with development of predator avoidance. The limitations in oxygen transport capabilities of amphibians and reptiles have forced a reliance on anaerobic pathways of respiration to support high-speed burst activity (*17*). Although caterpillars are also ectothermic and the major selective pressure shaping adaptive strategies of caterpillars is undoubtedly predation (*18*), their various adaptations to predators (including cryptic coloration, aposomatic coloration, urticating spines, and so on) are not related to locomotion. Physiologically, their locomotion is low performance (albeit costly) and entirely aerobic. There appears to be no selective advantage for high speed in caterpillars since they could not successfully outpace most of their predators and none of their parasitoids which rely on powered flight. Although it is perhaps not surprising that the energetics of caterpillar locomotion differ from those of animals with solid skeletons, given their developmental pattern, where body size changes more than 100-fold in a few short weeks, it is amazing that they are not more different.

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An Erythromycin Derivative Produced by Targeted Gene Disruption in *Saccharopolyspora erythraea*

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Derivatives of erythromycin with modifications at their C-6 position are generally sought for their increased stability at acid pH, which in turn may confer improved pharmacological properties. A recombinant mutant of the erythromycin-producing bacterium, *Saccharopolyspora erythraea*, produced an erythromycin derivative, 6-deoxyerythromycin A, that could not be obtained readily by chemical synthesis. This product resulted from targeted disruption of the gene, designated *eryF* (systematic nomenclature, *CYP107*), that apparently codes for the cytochrome P450, 6-deoxyerythronolide B (DEB) hydroxylase, which converts DEB to erythronolide B (EB). Enzymes normally acting on EB can process the alternative substrate DEB to form the biologically active erythromycin derivative lacking the C-6 hydroxyl group.

PREPARATION OF MODIFIED ANTIBIOTICS in cases where the parent molecule is complex and polyfunctional can be achieved by alteration of the antibiotic's biosynthetic pathway. Mutasynthesis (*1*) has been practiced for many years. More recently, genetic engineering has been useful in the formation of novel hybrid antibiotics (*2, 3*). We have used recombinant DNA technology to produce an erythromycin (Er) derivative that was not readily obtainable through chemical means but that was desired for pharmacological analyses.

Integrative plasmids have been used in *Saccharopolyspora erythraea* for targeted gene disruption in the elucidation of the organization of genes in the Er cluster (*4–6*). These plasmids, which carry small pieces of chromosomal DNA, are directed into the homologous site on the chromosome by single, reciprocal (Campbell) recombination. Frequently this process results in the generation of stable mutants that no longer produce Er. In this study, integrants were analyzed in a relatively uncharacterized region of the gene cluster between the *eryH* and *eryG* loci (*6*). Two strains so generated were distinguishable from the others by the production of a new metabolite, which was identified initially as a single spot by thin-layer chromatography (TLC) (Fig. 1).

One mutant was chosen for further analysis, and the new metabolite was isolated from its growth medium and purified (7). Fast atom bombardment mass spectrometry revealed that the metabolite had a molecular mass of 718 daltons, and ^{1}H and ^{13}C nuclear magnetic resonance (*8*) indicated that the structure 6-deoxyerythromycin (deoxy Er) A (Fig. 2A). Other components of the culture broth were separated and purified, and their structures were determined. They comprised the analogous biosynthetic congeners to Ers B, C, and D. A family of minor components, the 6-deoxy-15-norerythromycins (nor Ers) A, B, C, and D (Fig. 2A) was also isolated, separated, and characterized spectrally. These minor components presumably arose from the incorporation of acetyl coenzyme A (CoA), rather than of propionyl-CoA, as the starter unit to the polyketide synthase (*9*). The aglycone resulting from a similar aberration of polyketide biosynthesis, 8, 8α-deoxyoleandolide, has been isolated (*10*).

The mutagenic plasmids used to generate these strains were found through restriction analysis to contain fragments of 500 to 1000 bp from the *eryH-eryG* region. It was shown by DNA blot analysis that the plasmids had inserted into the homologous region of the chromosome (Fig. 3, A and B).

The locus disrupted by the mutagenic

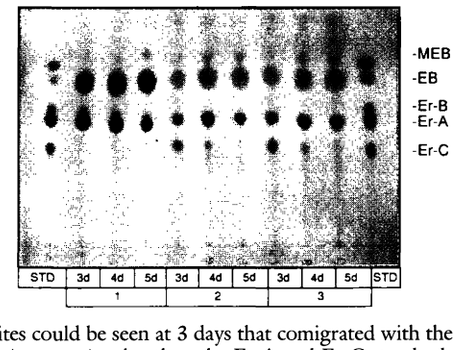

Fig. 1. Production of Ers by *S. erythraea* UW110 and by the insertion mutant, UW110::pMW56-H23, at 3, 4, and 5 days of growth, as analyzed by TLC. Lanes 1 (3d, 4d, and 5d), Er A–producing strain, UW110 (*met-4 leu-18 rif-63*), grown in T broth (7) at 32°C for 3, 4, and 5 days, respectively. The samples and chromatograph were prepared as described (*6*). Two major metabolites were visible, Er A and erythronolide B (EB). Lanes 2 and 3 (3d, 4d, and 5d): the mutant strain UW110::pMW56-H23, grown under the same conditions as the control strain, with thiostrepton selection (lanes 2), or without selection for the plasmid-borne *tsr* gene [thiostrepton (THIO) resistance] (lanes 3). Two major metabolites could be seen at 3 days that comigrated with the Er A and Er C standards, but that had a different (magenta) color than the Er A and Er C standards (STD). At 4 days and 5 days, the metabolite comigrating with Er C decreased in intensity, leaving only the spot comigrating with Er A. The results were not affected by thiostrepton selection. A light orange–colored spot was observed comigrating with EB, but did not appear in subsequent experiments and was not identified.

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