tions. Our model, of course, does not allow dissection of the magnitude of individual organ contributions or delivery of substrate limitations to the organs; however, the measured, integrated body rates are useful for investigating various pathophysiological processes on leucine metabolism as a whole. Finally, the stable isotope tracers permit the use of this approach in humans of all ages, and this scheme, with the choice of the appropriate model, may be applied to other amino acids.

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4. This model has been used previously with an L-[1-^{13}C]leucine tracer to measure the kinetic parameters Q_C, C, S, and B in Fig. 2 [see (*5*)].
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6. The infusion protocol consisted of administering intravenously a priming dose of sodium [^{13}C]bicarbonate (90 percent ^{13}C) and L-[^{15}N,1-^{13}C]leucine (99 percent ^{15}N, 92 percent ^{13}C) (0.08 mg/kg and 7.5 μmole/kg, respectively), and then infusing intravenously for 7 hours L-[^{15}N,1-^{13}C]leucine at the rate of 7.5 μmole per kilogram of body weight per hour. The fed subjects also received 0.08 g of protein per kilogram per hour from hourly meals (about 180 ml of Ensure, a milk and soya protein mixture from Ross Labs).
7. Plasma di- and monolabeled leucine enrichments were determined by selected ion monitoring–chemical ionization–gas chromatography–mass spectrometry [see (*8*)]. The dilabeled [^{15}N,1-^{13}C]leucine species was measured from the 218/216 molecular ion intensity ratio. Monolabeled leucine (either [^{15}N]leucine or [1-^{13}C]leucine) was measured from the 217/216 molecular ion intensity ratio. Total leucine ^{15}N enrichment (both [^{15}N,1-^{13}C]leucine and [^{15}N]leucine) was determined from the 129/128 fragment ion intensity ratio [the fragment does not contain the carboxyl-C; see (*8*)]. Total leucine ^{13}C enrichment (both [^{15}N,1-^{13}C]leucine and [1-^{13}C]leucine) was calculated from the three above-measured values. The dilabeled [^{15}N,1-^{13}C]leucine enrichment was used to calculate leucine N flux; the total leucine ^{13}C enrichment was used to calculate leucine C flux.
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Caterpillar Setae: Insulation for an Ectotherm

Abstract. *Gypsy moth caterpillars have long, soft setae distributed along the lateral portions of the body, but only short, stiff setae on the dorsal surface. Setae act as selective insulation for caterpillars by reducing the rates of convective heat exchange without affecting the rates of radiative heat exchange. Changes in posture abolish the effects of the setae by maximizing convection and minimizing radiant heat uptake.*

The importance of insulation to the heat balance of endotherms is well documented. It serves either as a means of conserving body heat (*1*) or as a radiation shield to prevent excessive radiant heating (*2*). Since insulation retards rates of heat exchange between the body and the environment, it would appear to be useless to small ectotherms, which must obtain all of their heat from the environment.

Although they are ectothermic, caterpillars routinely exhibit body temperatures far in excess of the air temperature (*3*) and are capable of thermoregulation by altering radiative and convective heat exchange through postural changes and movements in response to different microclimates (*4*). The present study demonstrates that insulation is significant to the thermal balance of gypsy moth caterpillars and that it differs in appearance and function from that of endotherms.

Late-instar gypsy moth (*Lymantria dispar*) caterpillars were supplied by the New Jersey Department of Agriculture Plant Pest Laboratory. The caterpillars, maintained in small plastic cups at room temperature, were fed a synthetic diet.

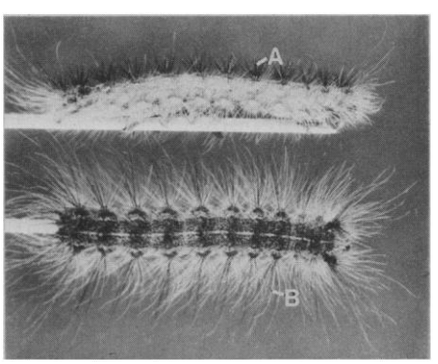

Fig. 1. Distribution of setae on gypsy moth caterpillars. (A) Short, stiff, spinelike setae on dorsal surface. (B) Long, soft setae in addition to short, stiff setae on lateral portion of the body.

Cooling curves for live and dead caterpillars in still air and at wind speeds of 1.0 and 2.0 m/sec were measured by suspending the caterpillars in a small wind tunnel (*5*). Before being placed in the wind tunnel, the caterpillars were heated to approximately 40°C with an incandescent light. They were then placed inside the tunnel and allowed to cool.

The effect of radiant heating on body temperature was measured by attaching caterpillars, with thermocouples inserted into the rectum, to wooden dowels (diameter, 2 mm) and exposing them to radiant heat loads (*6*) of 350, 700, and 975 W/m^2 from a 5000 K color temperature photoflood lamp (Kodak, FAY 650W).

Setae were removed from the caterpillars with forceps or jewelers' pliers. The setae were easily removed, and the caterpillars appeared to be unharmed by the procedure. Several caterpillars with setae removed pupated successfully and emerged into normal adults.

To determine the magnitude of heat loss by evaporation, we measured evaporative water loss of dead caterpillars with and without setae by weighing them before and after a 1-hour exposure at 35°C in dry air.

Setae occur in tufts, arising from setal stalks on tubercles located in distinct regions of each body segment (*7*). Two distinct types of setae occur, and different types do not always occur in the same location. Only short (mean length, 2.9 mm; N = 29), stiff setae arise from the dorsal tubercles (Fig. 1A). The largest outcropping of setae arise from the

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dorsolateral tubercles (Fig. 1B). In addition to the short, stiff setae, many long (mean length, 11.8 mm; $N = 31$) setae are also present. These undoubtedly increase the effective diameter of the body, which determines the convection coefficient (*8*). The body diameter of caterpillars without setae is only 7 mm compared with a width of about 30 mm measured from tip to tip of lateral setae.

In the absence of significant radiant heat, live or dead caterpillars cooled at the same rate. Cooling curves were linear when plotted on semilog coordinates (Fig. 2A). Therefore, cooling of caterpillars is not facilitated physiologically. In still air, and when they are oriented with the long body axis parallel to the prevailing wind direction, caterpillars with setae removed had cooling constants 30 to 45 percent greater than those for intact animals (Fig. 2B). Cooling constants increased with increasing wind speed for both intact caterpillars and those with setae removed. At a wind speed of 2.0 m/sec, the values of cooling constants were approximately twice that of the corresponding values in still air (Fig. 2B). When caterpillars were oriented with the long axis perpendicular to the prevailing wind (maximizing the effective surface area for convection), cooling constants for caterpillars with and without setae were not significantly different [mean ± standard deviation = 0.41 ± 0.04 ($N = 4$) and 0.44 ± 1 ($N = 4$), respectively] and were higher than those of caterpillars oriented parallel.

An increase in the rate of cooling when setae are removed (Fig. 2, A and B) can result from an increase in convection, an increase in evaporation, or both. At an ambient temperature (T_a) of 35°C, the evaporative water loss of caterpillars with setae removed was 31 percent higher than that of intact animals (16.5 compared to 12 mg/hour). However, the actual heat loss by evaporation does not account for the change in cooling constants. For a heat loss of 2.44 J per milligram of water evaporated, when the total body temperature (T_b) is 35°C (during cooling at a T_a of 20°C), the evaporative heat loss amounts to less than 4 percent of the total heat loss (*9*). A 30 percent increase in evaporation due to setae removal amounts to less than 6 percent of the total heat loss—about one-fifth of the total increase in cooling shown by caterpillars with setae removed. This is not surprising since the rate of evaporative heat loss for various insect species is smaller by one order of magnitude or more than the rate of heat exchange by convection (*10*). Therefore, we assume that the major effect of removal of setae on heat exchange occurs as a result of increasing convective heat exchange by reducing the effective body diameter.

Any advantage provided by the setae in reducing convection (Fig. 2, A and B) would be offset if the setae interfere with the uptake of solar radiation. Caterpillars with and without setae, oriented with the dorsal surface perpendicular to a radiant heat source (as would occur during basking), had steady-state body temperatures that were qualitatively similar and that were proportional to the radiation intensity (Fig. 2C). At radiation intensities between 350 and 975 W/m^2, the steady-state temperature excess ($T_b - T_a$) increased from about 6° to 16°C. Thus the animals with intact setae did not exhibit impaired ability to take up radiant heat in wavelengths approximating the solar spectrum. In animals whose long axes were oriented parallel to radiation in still air, the steady-state temperature excess was a least three times lower than those of animals oriented perpendicular to the radiation (Fig. 2C).

The insulation of caterpillars is quite different from that of endotherms for several reasons. In endotherms, heat is produced internally, and all body surfaces represent potential sites of heat loss. Consequently, the entire trunk of birds, mammals, and the thorax of endothermic insects is covered with thick, dense fur, feathers, or pile to retard rates of heat loss from the body surface. This is an effective mechanism for heat conservation that can be bypassed physiologically under conditions of heat stress by shunting warm blood to poorly insulated body regions (thermal windows). Since fur or feather insulation of endotherms retards rates of heat flow, it also serves to retard the rates of exogenous heat flow into the body. The high temperatures on the fur surface of camels and sheep during the day (*11*) and the contortions used by sunning roadrunners to take up solar radiation (*12*) are two examples of the effectiveness of fur and feathers as a radiation shield.

The morphology and physiology of caterpillars are sufficiently different from those of endothermic vertebrates and insects that if insulation is to occur at all,

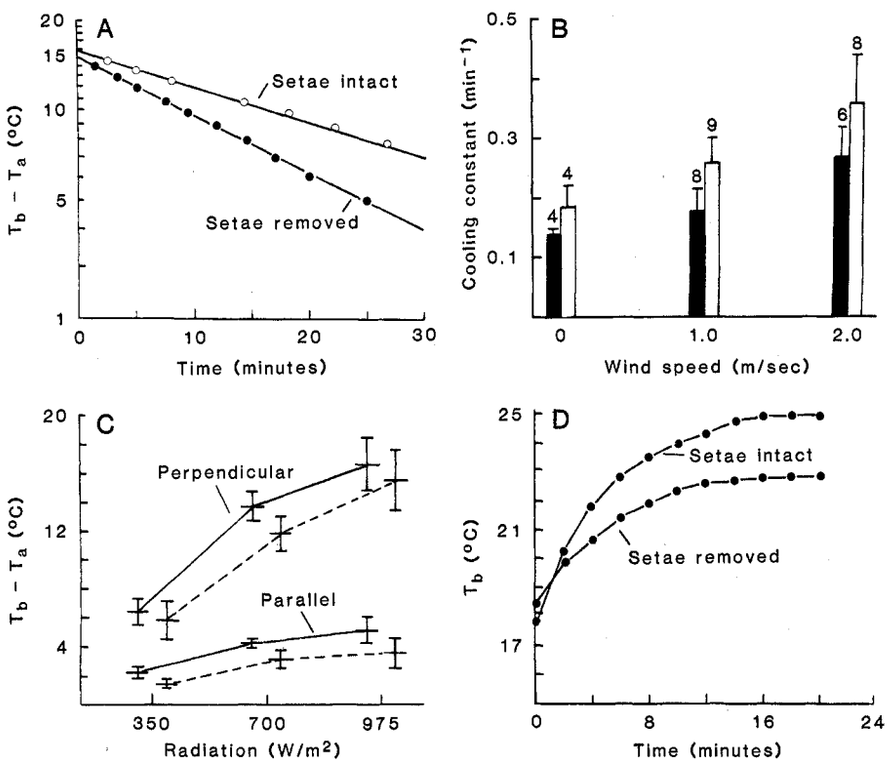

Fig. 2. Heat exchange and body temperatures of caterpillars under different environmental conditions. (A) Cooling curves of 1.85-g caterpillar with and without setae (wind speed, 2.0 m/sec). (B) The relation of cooling constants of caterpillar with setae (open bars) and without setae (closed bars) to wind speed in the absence of a radiant heat source. (C) The relation of steady-state temperature excess ($T_b - T_a$) to radiation intensity for caterpillars with setae (solid lines) and without setae (dashed lines). Upper curves represent caterpillars oriented with the long body axis perpendicular to a radiant heat source; lower curves represent caterpillars oriented with the long body axis parallel to a radiant heat source. (D) Heating curves and steady-state T_b of the same 1.75-g caterpillar, with and without setae, oriented with the long body axis perpendicular to a radiant heat source and parallel to the wind. Radiant intensity (700 W/m^2) and wind speed (1 m/sec) approximate midmorning conditions in the temperate zone.

it must have a somewhat different appearance and function. Since caterpillars are ectothermic, the major source of heat available to caterpillars is solar radiation. Therefore, evolving an insulation that reduces the rate of solar input would seem counterproductive. Also, since there is no large thermal gradient from one body region to another, thermal windows would appear to be useless to the caterpillar.

Nevertheless, the caterpillar setae serve as insulation by reducing the rate of convective heat exchange (Fig. 2A). Unlike insulation in endotherms, the capacity for radiant heat uptake is not affected by the setae. Therefore, a caterpillar having setae should absorb as much solar radiation as a smooth-skinned caterpillar of similar mass and body diameter, but should have lower rates of heat loss due to lower rates of convective heat exchange (Fig. 2D). Furthermore, the caterpillar can maintain a wide range of body temperatures simply by changing its orientation from parallel to perpendicular to solar radiation (Fig. 2C).

Why then, are not all terrestrial ectotherms "furry"? First, and perhaps most important, is the matter of size. The elongate shape of the caterpillar and its small body diameter relative to the size of the setae should maximize the effect of setae on heat exchange. For reptiles, which also control T_b by behavioral thermoregulation, the relative increase in body diameter caused by scales is small compared to the body diameter. Second, caterpillars are relatively sedentary and slow-moving, and therefore projections from the body surface should not interfere with movement. This may not be the case for relatively fast-moving terrestrial insects or reptiles. Other functions that setae perform in caterpillars might explain a selective advantage for their development before they had any significance in thermal balance. For example, setae are important for providing sensory information through tactile stimulation (*13*). In addition, since many setae are barbed (*7*), they serve as predator deterrents. Birds often refuse to feed on tent caterpillars (*Malacosoma disstria*), which have a similar distribution of setae as *L. dispar*, despite the fact that these caterpillars routinely feed in full sunlight and are more conspicuous than smooth-skinned caterpillars (*14*).

The contributing role of leaf boundary layers to convection needs to be assessed before a full understanding of steady-state T_b of caterpillars in the field is achieved. Nevertheless, regardless of their primary function and the selective pressures that caused them to arise, setae coupled with behavioral thermoregulation should be beneficial to caterpillars in maximizing T_b.

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Plasmid-Assisted Molecular Breeding: New Technique for Enhanced Biodegradation of Persistent Toxic Chemicals

Abstract. *The persistence of synthetic herbicides such as 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and its release in massive amounts as a herbicide (Agent Orange) have created toxicological problems in many countries. In nature, 2,4,5-T is slowly degraded by cooxidation and is not utilized as a sole source of carbon and energy. The technique of plasmid-assisted molecular breeding has led to the development of bacterial strains capable of totally degrading 2,4,5-T by using it as their sole source of carbon at high concentrations (greater than 1 mg/ml). Spectrophotometry and gas chromatography reveal various intermediates during growth of the culture with 2,4,5-T.*

During the past several decades, the release of various synthetic chemicals—mostly chlorinated aromatics—into the environment has resulted in serious environmental pollution (*1*). The problem is not only the toxicity of the chemicals, but their persistence, so that they ultimately contaminate human bodies (*2*). An example of such a toxic chemical is the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), which is often suspected to exert genotoxic effects; in particular, it is suspected of causing certain birth malformations in humans (*3*). Natural microflora degrade 2,4,5-T very slowly by cooxidative metabolism (*4*). Bacteria metabolizing 2,4,5-T and other recalcitrant compounds do not increase in cell number and seldom incorporate the carbon derived from the cooxidative metabolism of these compounds into their cell mass (*5*). The persistence of these compounds is therefore due to an inability of natural microbial flora to degrade them totally in order to derive their carbon and energy from the process.

We have recently reported the occurrence of plasmids that specify total degradation of chlorinated aromatic compounds such as 3-chloro- or 4-chlorobenzoic acid (*6*). We have demonstrated that the plasmid pAC25, which encodes a complete 3-chlorobenzoate degradative pathway, does not allow the host cells to utilize 4-chlorobenzoate; however, introduction of the TOL plasmid (specifying xylene and toluene degradation) into such a cell provides a broad substrate-specific benzoate oxygenase, which helps in the conversion of 4-chlorobenzoate to 4-chlorocatechol (*7*). 4-Chlorocatechol can then be completely metabolized by the pAC25-specified enzymes. Similarly, introduction of the TOL plasmid into the cells harboring the pAC25 plasmid and continued selection on 3,5-dichlorobenzoate allow the emergence of cells that can also utilize 3,5-dichloro-

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