volve "choice tests" during which the animals are allowed to investigate two stimulus plates, each carrying a different type of scent. Hansen scores for marking, sniffing, and contacts are computed and analyzed by nonparametric statistics. A statistically significant preference for one class of mark over another (such as male over female) indicates that information for discriminating these two classes is found in the mark [S. Siegel, *Nonparametric Statistics* (McGraw-Hill, New York, 1956].
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12. The Monell Center maintains a successful breeding colony of *Saguinus fuscicollis*. Our resident population of 150 animals lives in family groups or pairs in wire mesh cages equipped with natural branches and sleeping boxes. Visual contact is limited by solid metal walls on two sides of each cage to avoid aggressive arousal in these easily stressed animals.
13. Material was collected by allowing the monkey to scent mark a 60-cm-long frosted glass plate. The animals are accustomed to this procedure and usually mark within seconds of introducing the plate into their home cage. After collection, each scent-marked plate was rinsed with 30 ml of a solution containing methylene chloride and methanol in a ratio of 3 to 1. For gas chromatographic analysis, the solvent mixture was removed by rotary evaporation at reduced pressure; the residue was then dissolved in a small amount of hexane and evaporated under nitrogen to a suitable volume (~15 μl).
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Flight of Winter Moths Near 0°C

Abstract. *Some noctuid winter moths fly at near 0°C by maintaining an elevated (30° to 35°C) thoracic muscle temperature. Geometrid winter moths sustain themselves in free flight at subzero muscle temperatures. However, the temperature characteristics of citrate synthase and pyruvate kinase from both of these different kinds of moths and from a sphinx moth that flies with a muscle temperature of 40°C are nearly identical. Furthermore, mass-specific rates of energy expenditure of both kinds of winter moths are also similar at given thoracic temperature (near 0°C). The geometrids that are able to fly with a thoracic temperature near 0°C do so largely because of unusually low wing-loading, which permits a low energetic cost of flight.*

Most moths that have been investigated are highly endothermic during flight (*1, 2*), and some species maintain a thoracic temperature (T_{Th}) near 45°C (*3*), up to 35°C above ambient temperature (T_A). The endothermic moths shiver (*4*) before flight until the power output of the muscles is sufficient for takeoff (*5*). The lower the T_A, the more energy the moths must expend for any given preflight warm-up or the more heat they must retain in their flight muscles to continue flight, or both (*6*). We investigated the question of why certain noctuid and geometrid moths are able to fly in the winter at zero or even subzero temperatures.

In one group of winter moths, the Cuculiinae (Noctuidae), the adults emerge in the fall (*7*) and fly throughout the winter when T_A approaches 0°C. Some of these moths (*Eupsilia morrisoni, E. tristigmata, E. sidus*, and *E. vinulenta*) were captured with sugar bait (*8*) in Maine and Vermont in February, and they warmed up by shivering in the laboratory at T_A as low as −3°C. They continued to shiver until T_{Th} exceeded 30°C (*9*), their minimum T_{Th} for sustained flight. Thoracic temperatures were maintained between 30° and 39°C during continuous free flight at T_A from 3° to 21°C (Fig. 1). Prolonged shivering at low T_A (*10*), a 1.0-mm-thick layer of insulating pile on the thorax, and greatly reduced heat flow from the thoracic muscles to the abdomen (*11*) allow these moths to resist the cold.

A second group of winter moths (Geometridae) also emerge in the fall. We examined two species, *Operophtera bruceata* Hulst. and *Alsophila pometaria* Harris that fly in late November (in northeastern United States) until the first heavy snowfalls stop their activity. In these moths the females are wingless, and the males (which do not feed) fly both at night and in the daytime. We found *O. bruceata* flying in the field in daytime at T_A as low as −3°C.

The winter geometrids are small moths (usually <10 mg) and they are uninsulated. They were at no time observed to either shiver or bask. Thoracic temperatures of flying moths were within one Celsius degree of T_A (Fig. 1). What allowed these insects to fly with a muscle temperature below the freezing point of water? Did they have specialized enzyme systems that operate at subzero temperatures? Many insects survive such temperatures by becoming inactive and adapting biochemically (*12*). But to our knowledge no insect has been shown to operate its wings in flight while its thoracic muscles are cooled to 0°C or lower.

We analyzed (*13*) the catalytic efficiency of citrate synthase (E.C. 4.1.3.7) in noctuid and geometrid winter moths over temperature (Fig. 2). Overall activities of the enzyme per gram of thorax fell into a common range (50 to 100 units, measured at 15°C), even though the noctuids flew with a T_{Th} near 35°C, while the geometrids flew with a T_{Th} as low as −3°C. Similarly, the thermal response of the enzyme in a sphinx moth, *Manduca sexta*, which normally flies with a T_{Th} near 40°C (*6*), also fell into the same range. These results contrast with the available data in other ectotherms, which show a pronounced increase in enzyme activity associated with decreasing temperature (*14*).

Enzyme activities in all moth species that we examined revealed identical behaviors over a range of temperatures, surprisingly independent of the T_{Th} necessary for flight. All data points fall on lines with slopes that are not significantly different from each other, giving the four moths enzymes with equal energies (E_a) and enthalpies ($\Delta H^{\ddagger}$) (*15*) of activation over the entire temperature range (E_a = 10,140 cal mol^{-1}; standard devi-

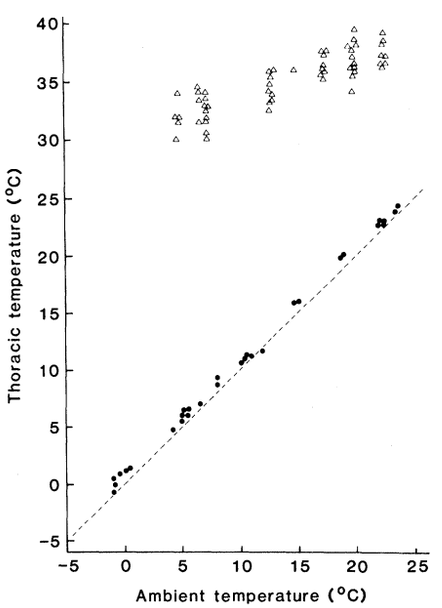

Fig. 1. Thoracic muscle temperatures of *Eupsilia* spp. (△) and *Operophtera bruceata* (●) in free flight, as a function of ambient temperature. Dashed line indicates the isothermal. Note that *O. bruceata* flies with thoracic temperature close to ambient temperature down to −3°C. The thoracic temperatures indicated also reflect the range of ambient temperature over which the moths were able to remain in continuous free flight.

ation, ±310 cal mol^{-1}). The same holds true for thorax pyruvate kinase (E.C. 2.7.1.40) between 5° and 35°C, giving a common $\Delta H^{\ddagger}$ of about 12,200 ± 680 cal mol^{-1} at 25°C.

Although mitochondrial enzymes tend to be more conservative in their characteristics than their cytoplasmic counterparts (*16*), enthalpy compensation is a common phenomenon in low-temperature-adapted ectotherms (*15, 17, 18*). However, this type of adaptive strategy apparently does not apply to the diverse kinds of moths that we examined. Furthermore, the enzymes from the highly endothermic *M. sexta* are not adversely affected in their catalytic efficiency by temperatures as low as 5°C where mammalian enzymes (*19*) can be rendered ineffective because of low-temperature-induced loss in quaternary structure (*20*). Continuous Arrhenius plots for both citrate synthase (Fig. 2) and pyruvate kinase suggest that different forms of the enzymes are not involved in metabolic regulation during temperature transition.

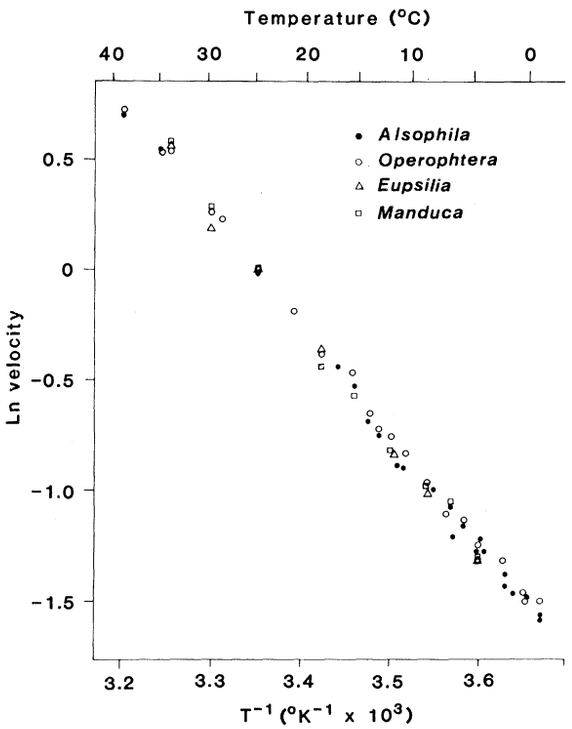

Fig. 2. Temperature dependence (Arrhenius plot) of citrate synthase reaction in four species of moths. The slopes of the regression lines (E_a) are identical for the enzyme over a temperature range from below 0°C to above 35°C. Units of enzyme activity (micromoles of product per minute per gram of thorax at 25°C ± standard deviation) and energies of activation (slopes in arbitrary units including 95 percent confidence interval) are: *Alsophila pometaria*, 116 units ± 31 (eight determinations), slope, −5073 (−5234, −4909; 22 observations); *Eupsilia morrisoni*, 52.5 units ± 10.1 (n = 6), slope, −5056 (−5498, −4614; n = 7); *Manduca sexta*, 46.0 units ± 14 (n = 4), slope, −5312 (−5675, −4948; n = 9); *Operophtera bruceata*, 70.5 units ± 26 (n = 8), slope, −5118 (−5346, −4890; n = 22). Activities for all species are normalized for 15°C to give an arbitrary unit activity.

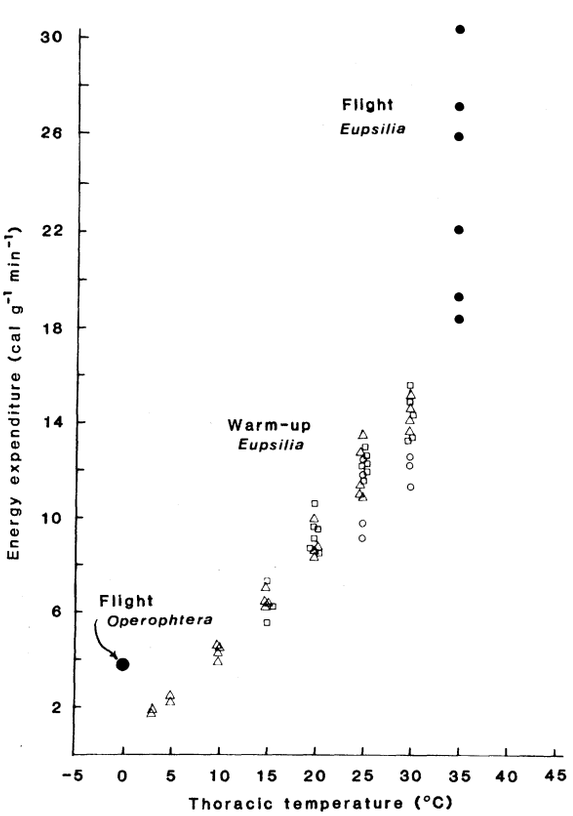

Fig. 3. Rates of energy expenditure in *Eupsilia* spp. during preflight warm-up from ambient temperatures of 0°C (△), 10°C (□), and 20°C (○), calculated from thoracic temperature (*21*) and measured rates of flight metabolism (*22*) at T_{Th} near 35°C (●). Symbol at 0°C includes calculated energy expenditure of flying *Alsophila* and *Operophtera*, as determined from measured thoracic temperatures (Fig. 1) and regression of conductance for geometrid moths (*2*).

How do the minimum rates of energy expenditure needed to support flight compare with the apparent lack of thermal adaptation in the moth's enzymes supporting flight? We determined rates of energy expenditure in the noctuid moths as a function of T_{Th} at T_{Th} below the minimum for flight from data on changes in T_{Th} during preflight warm-up (*21*). As in other endothermic moths (*5*), these rates were strongly constrained by temperature. At a T_{Th} of 5°C, for example, energy expenditure was 2 cal per gram of thorax per minute, but at a T_{Th} of 30°C, it had increased to 12 to 16 cal per gram of thorax per minute, a value close to that needed to support free flight (*22*).

Technical difficulties preclude at present the direct measurement of energy expenditure during free flight of the tiny geometrid moths that do not hover in a respirometer. But, with our data on thoracic temperature in flight (Fig. 1) and the equation for mass-specific conductance of geometrid moths (*23*), we were able to estimate an energy expenditure during flight. These estimated values for both *Operophtera* and *Alsophila* indicate that flight in these insects must involve a remarkably low rate of energy expenditure (*23*). Indeed, even with a thoracic muscle temperature of 0°C the moths support flight with an energy cost near 4 cal per gram of thorax per minute, which is similar to the energy output of shivering in *Eupsilia* spp. at a T_{Th} of 10°C (Fig. 3). The metabolic rate sufficient to keep the geometrids in flight, however, is only one-tenth that required to support flight in *Eupsilia*. We conclude, however, that the metabolic rates of the geometrids and noctuids are similar at given low T_{Th}, and this conclusion is valid even if we assume a large error in our calculated costs of flight in the geometrids.

How can the geometrids support flight at low energetic cost? Energy expenditure in flight as well as preflight warm-up is a function of wing-beat frequency, which is limited by muscle temperature (*5*). The regression equations for wing-beat frequency (T_A, 11° to 22°C) indicate that *Eupsilia* spp. and *Operophtera* and *Alsophila* have wing-beat frequencies of 59.4 and 4.0 to 4.1 Hz, respectively (*24*).

At a T_A of 0°C, however, the wing-beat frequency of the temperature-conforming geometrids should be decreased considerably. If the geometrid wing-beat frequency is halved at 0°C, it would be approximately 30 times lower than that of the noctuids.

Geometrids and other nocturnal nonfeeding Lepidoptera generally have large wings (*1*) relative to their mass (low wing loading), and this characteristic confers a low energetic cost of flight (*25*). The mean wing-loading in *Eupsilia* sp. was 43 mg/cm^2 (*24*). In the winter-flying *Operophtera* and *Alsophila*, however, wing-loading (3.20 mg/cm^2 and 3.90 mg/cm^2) is not only 10 to 14 times lower than in the noctuids but is also lower than in all other geometrid (and other moth) species from both tropical (*1, 26*) and temperate regions (*2*) that have been examined. Low wing-loading that decreases the cost of transport may be a preadaptation in these geometrids that has allowed them to fly at low ambient and muscle temperatures. Our findings suggest that although morphology affecting thermoregulation can vary radically, the adaptations at the enzyme level for temperature are apparently highly conservative.

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9. Body temperatures during preflight warm-up were measured with 0.001-mm diameter copper-constantan thermocouples inserted approximately 1 mm into the thorax. Thoracic and ambient temperatures were each recorded at 10-second intervals with a Honeywell potentiometric recorder. All temperatures were referenced to a National Bureau of Standards thermometer. For moths in flight, thoracic temperature was measured after the animal had been in continuous flight for sufficiently long to ensure equilibration of body temperature (>1.5 minutes). Temperature measurements were made with a Bailey Bat-12 digital thermocouple thermometer [B. Heinrich and M. J. Heinrich, *Physiol. Zool.* **56**, 552 (1983)]. Measuring artifacts of temperature in the small moths were corrected as previously described by B. Heinrich and K. Pantle [*J. Exp. Biol.* **62**, 599 (1975)].
10. At a T_A of 20° and 0°C, for example, the moths shivered for 1.0 to 23 minutes, to achieve a flight temperature of 32° to 35°C.
11. During preflight warm-up abdominal temperature did not exceed 0.5°C above T_A, and during flight it did not exceed 3°C above T_A.
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13. After capture, moths were quick-frozen on dry ice and stored for a maximum of 4 weeks at −80°C. Tissues from three to six specimens of the geometrid moths (*Alsophila* or *Operophtera*) were pooled, although specimens of the other two species were analyzed individually. Thoraxes, thawed and carefully freed of legs, wings, and hair, were homogenized and sonicated extensively in 20 volumes of imidazole buffer (50 m*M* imidazole-HCl, containing 10 m*M* glycerol, *p*H 7.4 at 20°C). Homogenates, prepared on ice, were centrifuged at 6000*g* for 20 minutes at 0°C. Resulting supernatants were kept on ice and diluted with buffer. Enzyme activities were measured spectrophotometrically [T. P. Mommsen, C. J. French, P. W. Hochachka, *Can. J. Zool.* **58**, 1785 (1980)]. All assays were performed in duplicates or triplicates (temperatures below 10°C), and cuvette temperature was monitored before and after incubation. One unit is defined as the amount of enzyme forming 1 μmol of product per minute under saturating conditions.
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20. In vivo, however, secondary effects of changes in membrane fluidity with decreasing T_A may alter enzyme catalysis over and above direct thermodynamic considerations [M. Sinensky, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 522 (1974)]. Enzyme-level adaptation through a compensation in the entropy term is an interesting possibility (*17*), but one beyond the scope of our study.
21. Rates of heat production were calculated from passive rates of heat loss in dead animals and heat storage during T_{Th} increase in live animals during shivering (*5*).
22. Oxygen consumption of flying moths (*Eupsilia* spp.) was measured with an S-3A Applied Electrochemistry oxygen analyzer system measuring to 0.01 percent O_2 concentration. The moths were flown for timed durations (2 to 15 minutes) in a 1680-ml respirometer, and O_2 content of the air was determined immediately before and after each flight.
23. Solving the equation of Casey and Joos (*2*) for conductance in geometrids (log C = −0.61 log mass + 3.01) indicates that the *Operophtera* and *Alsophila* with mean mass of 9.8 mg and 8.6 mg should expend 3.68 and 3.95 cal per gram of body weight per minute, respectively, to maintain a T_{Th} of 0.5°C above T_A during flight at approximately 0.5 m/second.
24. The regression equation of Casey and Joos (*2*) for wing-beat frequency for geometrids is: log n = −0.025 + 0.492 log m + 0.133 log ℓ + 0.255 log $w\ell$, where n = wing-beat frequency per second, m = mass in milligrams, ℓ = wing length in centimeters, and $w\ell$ = wing loading in milligrams per square centimeter. Mean values ($n \geq 10$) for *Operophtera*: m = 9.8 mg, ℓ = 1.38 cm, and $w\ell$ = 3.20 mg/cm^2. Mean values for *Alsophila*: m = 8.64 mg, ℓ = 1.23 cm, and $w\ell$ = 3.90 mg/cm^2. The corresponding equation for noctuid moths is: log n = 1.224 + 0.849 log m − 1.39 log ℓ − 0.643 log $w\ell$; mean values for *Eupsilia* spp. were: m = 164 mg, ℓ = 1.58, and $w\ell$ = 43 mg/cm^2.
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27. We thank D. Schweitzer and J. A. Franklemont for valuable discussion and for identifying the moths. Supported by NSF grant DEB-816662 to B.H. and by an NSERC (Canada) operating grant to T.P.M.

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Mammalian and Yeast *ras* Gene Products: Biological Function in Their Heterologous Systems

Abstract. *Activated versions of* ras *genes have been found in various types of malignant tumors. The normal versions of these genes are found in organisms as diverse as mammals and yeasts. Yeast cells that lack their functional* ras *genes, RAS^{SC}-1 and RAS^{SC}-2, are ordinarily nonviable. They have now been shown to remain viable if they carry a mammalian* ras^H *gene. In addition, yeast-mammalian hybrid genes and a deletion mutant yeast RAS^{SC}-1 gene were shown to induce morphologic transformation of mouse NIH 3T3 cells when the genes had a point mutation analogous to one that increases the transforming activity of mammalian* ras *genes. The results establish the functional relevance of the yeast system to the genetics and biochemistry of cellular transformation induced by mammalian* ras *genes.*

The *ras* genes constitute a multigene family that is highly conserved among eukaryotes, including mammals and yeast. These genes and their 21-kD protein products (p21) were first identified as the viral oncogenes of Harvey and Kirsten murine sarcoma viruses (Ha-MuSV and Ki-MuSV, respectively) (*1*).

High level expression of a normal (nonmutated) mammalian p21 *ras* protein can induce tumorigenic transformation of established rodent cells. However, forms of the protein encoded by *ras* genes that contain certain point mutations can induce these tumorigenic changes with much greater efficiency. Mutated forms of cellular *ras* oncogenes have been implicated in the pathogenesis of several human cancers. Activating point mutations that lead to a more highly oncogenic form of mammalian p21 protein, which are present in v-*ras* (viral) and c-*ras* (cellular) oncogenes isolated from tumor cells, include those that substitute any one of several amino acids for Gly^{12} or Gln^{61} or that substitute Thr^{59} for the normal Ala^{59}. Addition of an enhancer element to these structurally altered *ras* genes can enable them to transform primary rodent cells directly (*1, 2*).