of wind pollination mechanisms would result in minimal pollen deposition. Amazon lake mud, however, turns out to have high pollen concentrations, consistent with the high pollen influx (20,000 grains cm^{-2} $year^{-1}$) measured in our airborne-pollen traps from Ecuadorian Amazonia, from near Manaus, and from the coastal rain forest of eastern Brazil [M. B. Bush, *J. Veg. Sci.* **3**, 275 (1992)]. The Amazon pollen assemblies are extremely diverse by temperate standards (we recognized 169 taxa in the Lake Pata analyses, based on our reference collection of >5000 neotropical species in >1500 genera. This high influx of diverse pollen taxa certainly holds a remarkably detailed history of Amazonian forest associations, the full potential of which will be realized only when more autecological information is available.

18. Forest signatures are also inherent in the percentages of the more copious pollen-producing families in the forest such as Moraceae, Urticaceae, Melastomataceae, and Myrtaceae. If these percentages, together with the percentages of wind-blown pollen of grasses, are used, it is possible statistically to separate the principal plant associations of the lowland neotropics, providing an independent identification of DTRF in the Lake Pata pollen spectra [M. B. Bush, *Holocene* **1**, 162 (1991)].
19. Pollen counts at coarse intervals reveal similar forest spectra throughout the bottom 5 m of the section also, suggesting that DTRF was the local vegetation throughout the complete glacial cycle.
20. *Podocarpus* pollen has never been recorded in more than trace amounts in surface samples or Holocene sections from lowland forests of the Neotropics (*22*). The few grains of *Podocarpus* in surface and Holocene records can best be explained as the result of wind transport over long distances from *Podocarpus* stands at high elevations in the Andes and elsewhere or from rare *Podocarpus* trees in gallery forests [M. L. Salgado-Labouriau, *Proceedings of the International Conference on Aerobiology*, Berlin (1978), p. 89. Our 3 years of pollen trap data (100 traps) in Ecuadorian Amazonia include almost no *Podocarpus*. Traps in a 1-ha plot of coastal rain forest of southeastern Brazil (1 year of data, H. Behling, unpublished data) yielded only 0.8% *Podocarpus* despite the fact that three *Podocarpus* trees grow within the plot. Thus, *Podocarpus* populations much denser than those now found in Neotropical lowlands would be required to account for high *Podocarpus* pollen percentages. All reports of significant *Podocarpus* populations in northwestern Brazil are from Pico da Neblina, a mountain rising to 3014 m, 90 km northeast of the Hill of the Six Lakes. Principal herbarium holdings from the region are at the Field Museum and the New York Botanic Garden, both of which we searched for *Podocarpus*, finding minimum recorded elevations as follows: *P. bucholzii*, 2100 m; *P. magnifolius*, 1725 m; *P. roraimae*, 1200 m; *P. steyermarkii*, 1200 m; and *P. tepuiensis*, 1100 m. If the Lake Pata *Podocarpus* pollen represents descents of these populations, minimal descents were 800 m to the summit of the Hill of the Six Lakes and 1000 m to the surrounding lowlands.
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Achievement of Thermal Stability by Varying Metabolic Heat Production in Flying Honeybees

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Thermoregulation of the thorax allows endothermic insects to achieve power outputs during flight that are among the highest in the animal kingdom. Flying endothermic insects, including the honeybee *Apis mellifera*, are believed to thermoregulate almost exclusively by varying heat loss. Here it is shown that a rise in air temperature from 20° to 40°C causes large decreases in metabolic heat production and wing-beat frequency in honeybees during hovering, agitated, or loaded flight. Thus, variation in heat production may be the primary mechanism for achieving thermal stability in flying honeybees, and this mechanism may occur commonly in endothermic insects.

Like many other large endothermic insects, honeybees regulate thoracic temperatures relatively closely over a range of air temperatures (*1–4*). Thoracic thermoregulation in flying honeybees is thought to occur primarily through varying evaporative heat loss, which is made possible by the extrusion of nectar carried during foraging (*1–5*). Thermoregulation during flight by varying heat production has been considered implausible for endothermic insects because metabolic rates increase with rising thoracic temperature during warm-up (*1*) and because the power output required during flight has been considered to be determined by aerodynamic rather than thermoregulatory needs (*1, 2, 6*).

We discovered evidence for a thermal effect on the metabolic rate of honeybees in high-intensity, agitated flight during a study of African, European, and hybrid honeybees (*7*). In these assays, bees were agitated continuously during the measurements to elicit high-intensity flight. Both flight metabolic rate and wing-beat frequency were negatively correlated with air temperature (*8*). Flight metabolic rates were 25% lower at 30°C than at 20°C, with air temperature accounting for 47% of the measured variation in metabolic rate and 12% of the variation in wing-beat frequency over this small thermal range, despite the genetic diversity of the bees (Fig. 1). For these analyses, data from African, European, and hybrid colonies were pooled, because genotype did not significantly affect the slope of the regression relations between air temperature and metabolic rate or wing-beat frequency.

We conducted an experimental test of these correlative data by flying European honeybees at a range of air temperatures within a temperature-controlled room at the apiary of the University of California, Davis. Outgoing foragers were collected from two colonies, and flight metabolic rates, wing-beat frequencies, and thoracic and abdominal temperatures were measured (*8–10*). Honeybee thoracic temperature varied much less than ambient temperature (Fig. 2). Abdominal temperatures closely tracked air temperatures (Fig. 2), supporting previous findings that variable heat transfer between thorax and abdomen is not an important mechanism of thermoregulation in flying honeybees (*1, 3–5*). Metabolic rates of flying agitated bees decreased by 50% as air temperature rose from 20° to 40°C (Fig. 3A). Metabolic rates were unaffected by nectar loads greater than 50% of body mass at either 21° or 38°C (*11*) (Table 1), suggesting that agitated honeybees fly at near-maximal performance (*7*) and that loaded bees also thermoregulate by varying metabolic heat production.

Variation in metabolic rates with temperature for the agitated bees, which fly rapidly and erratically about the respirometry chamber, might reflect varying degrees of agitation and intensity of flight performance. Hovering flight is considered to be a well-defined behavior, in which metabolic rate is determined solely by the aerodynamic power requirements for hovering (*1, 2, 5*). We tested the effect of air temperature on the metabolic rate of bees in undisturbed hovering flight, with the expectation that the metabolic rate during hovering would be independent of air temperature (*12*). However, the metabolic rates of honeybees in stationary, undisturbed hovering flight decreased by 40% as air temperature rose from 20° to 40°C (Fig. 3A). Heinrich measured flight metabolic rates for hovering honeybees that were 20 to 40% below ours and independent of air temperature (*4*). However, by using a flow-through respirometry sys-

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tem, we measured metabolic rates over a much shorter time period than Heinrich (*4*), perhaps allowing evaluation of more continuous, vigorous flight behavior. The large sample sizes (more than 900 bees), the repeatability of the results with different techniques and populations, and the correlated effects of temperature on metabolic rate and wing-beat frequency confirm that a rise in air temperature from 20° to 40°C strongly decreases flight metabolic rate in honeybees.

The finding that metabolic rate can vary while bees perform what appears to the human eye to be similar behaviors poses an important challenge for our understanding of insect flight. The variable metabolic rates during hovering flight at different air temperatures could occur by a change in the efficiency of conversion of metabolic power to mechanical power or by variation in mechanical power output. Changes in the efficiency of producing mechanical power from metabolic power with air temperature might occur as a result of effects of wing-stroke frequency or amplitude on the efficiency of muscle, elastic energy storage, or aerodynamic power production, or by effects of thoracic temperature on the magnitude of elastic energy storage.

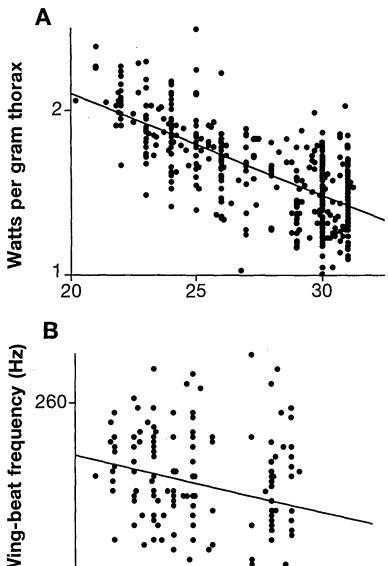

Fig. 1. The relation between air temperature and flight metabolic rate (**A**) and wing-beat frequency (**B**) of African, European, and hybrid honeybees in agitated flight. (A) The equation for the least squares linear regression line is: watts per gram thorax = 3.32 − (0.061 × air temperature) (°C, $F_{1,313}$ = 275, $P < 0.001$; standard error of slope = 0.0036, of intercept = 0.100). (B) The equation for the least squares linear regression line is: wing-beat frequency (Hz) = 281 − (1.45 × air temperature) (°C, $F_{1,129}$ = 18.1, $P < 0.001$; standard error of slope = 0.341, of intercept = 9.3).

Changes in mechanical power during hovering might occur in a manner analogous to high- and low-intensity modes of treading water in humans, with variation in mechanical power output being relatively uncoupled from movements of the body's center of mass. In our experiments, the correlated 50% change in metabolic rate and 16% change in wing-beat frequency between 20° and 40°C (Fig. 3, A and B) are consistent with the theoretical prediction that metabolic rate should be approximately proportional to the cube of wing-beat frequency (*13*), suggesting variation in mechanical power output with temperature. However, because power output during flight depends on multiple factors (*13, 14*), further experiments will be necessary to determine the mechanisms whereby metabolic heat production varies with air temperature

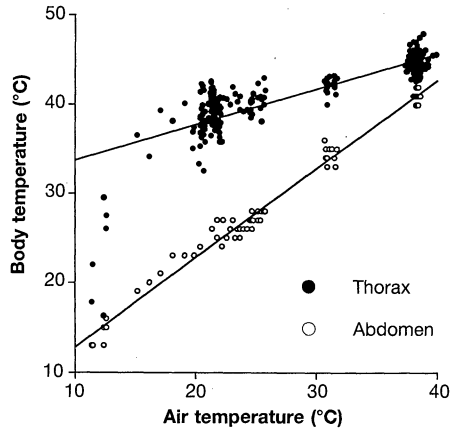

Fig. 2. Effect of air temperature on the thoracic (●) and abdominal (○) temperatures of agitated, flying European bees. The equation for the least squares linear regression line presented for thoracic temperatures is: thoracic temperature = 29.6 + (0.40 × air temperature) (°C, r^2 = 0.68, $F_{1,293}$ = 622, $P < 0.001$; standard error of slope = 0.016, of intercept = 0.50). The equation for the least squares linear regression line presented for abdominal temperatures is: abdominal temperature = 2.8 + (1.00 × air temperature) (°C, r^2 = 0.99, $F_{1,99}$ = 8690, $P < 0.001$; standard error of slope = 0.011, of intercept = 0.33).

Table 1. Effect of air temperature and loading with artificial nectar on the body masses, metabolic rates (milliwatts per bee), and thoracic temperatures (°C) of agitated, flying honeybees (mean ± SEM, n = 7 to 9 at each value).

21°C		38°C	
Loaded	Unloaded	Loaded	Unloaded
Body mass (mg)			
133 ± 3.4	87 ± 3.0	141 ± 3.1	84 ± 4.4
Metabolic rate (milliwatts)			
53 ± 2.5	56 ± 2.3	26 ± 2.6	25 ± 2.5
Thoracic temperature (°C)			
38 ± 0.6	39 ± 0.3	44 ± 0.3	44 ± 0.3

during hovering.

Variation in metabolic heat production is likely to be the major mechanism whereby thermal stability is achieved in hovering honeybees, because the 50% decrease in metabolic heat production between 20° and 40°C is similar to the 40% decrease in the elevation of thoracic above air temperature (Figs. 2 and 3A), and we never observed nectar regurgitation in these bees. Our results are also supported by field studies which have demonstrated that higher air temperatures are correlated with lower wing-beat frequencies in free-flying honeybees (*15*). To our knowledge, this is the first direct demonstration that thermal stability can be achieved by varying heat production in a free-flying endothermic insect. The decrease in metabolism with increasing temperature may be actively controlled by a homeostatic mechanism or may occur secondarily as a result of changes in muscle

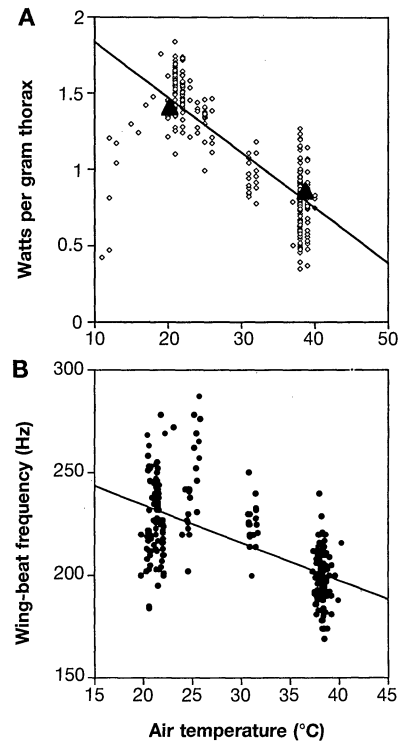

Fig. 3. (**A**) Effect of air temperature on flight metabolic rate of agitated, flying European bees (◇, n = 299) and European bees in undisturbed, hovering flight (▲, standard errors within the symbols, n = 33 at 20°C and n = 16 at 38°C). The line shown indicates the least squares regression line for agitated flying bees [watts per gram thorax = 2.20 − (0.036 × air temperature)] (°C, r^2 = 0.66, $F_{1,294}$ = 573, $P < 0.001$; standard error of slope = 0.0015, of intercept = 0.047). (**B**) Effect of air temperature on the wing-beat frequency of agitated, flying European honeybees. The equation for the regression line shown is: wing-beat frequency = 271 − (1.84 × air temperature) (°C, r^2 = 0.47, $F_{1,270}$ = 199, $P < 0.001$; standard error of slope = 0.131, of intercept = 4.1).

efficiency or because of direct inhibitory effects of high thoracic temperatures on the flight musculature. Force production by tethered honeybees decreases by 45% as thoracic temperature rises from 39° to 45°C (*16*), similar to the decrease in metabolic rate we observed over the same thoracic temperature range (Figs. 2 and 3A).

Variation in heat production may explain thermal stability during flight in other endothermic insects and potentially could contribute to thermoregulation in birds (*17*). Although heat production does not vary with air temperature in moths or bumblebees, which can shunt heat to the abdomen to increase heat loss (*1*), many endothermic insects lack the capacity to modulate heat transfer between thorax and abdomen (*1, 2*). Wing-beat frequencies and flight metabolic rates have also been reported to decrease with air temperature in bees of the genus *Centris* (*18, 19*). Wing-beat frequencies and inferred heat production decline at higher air temperatures in the dragonfly *Anax junius* (*20*), suggesting that the role of varying heat production in achieving thermal stability during flight may deserve a general reevaluation for large endothermic insects. For insects in which flight metabolic rate varies with air temperature, it will be important to reexamine energetic models of migration, foraging, and mating for temperature sensitivity.

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9. After completion of the metabolism measurements, bees were shaken out of the respirometry chamber into a plastic bag and restrained against a plastic foam surface. Thoracic and abdominal temperatures were measured within 10 s of flight by inserting a Physitemp model MT-29/1 hypodermic microprobe (time constant, 0.025 s) into first the thorax and then the abdomen.
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11. Loaded bees were collected after feeding ad libitum from a 1 M sucrose solution. Unloaded bees were collected as they arrived at the feeder. Metabolic rates during agitated flight were measured as described (*7, 8*).
12. Metabolic rates of hovering European honeybees from a single colony at Arizona State University, Tempe, were measured in a vertical Plexiglas cylinder (internal diameter, 51 mm) by measuring carbon dioxide emission by flow-through respirometry essentially as described (*7, 8*), except that flow rates through the chamber averaged 2 to 4 liter min^{-1}. Data are reported only for the small fraction of bees that hovered without any provocation, in the center of the tube, with less than 1 cm of verticle movement during the respirometry measurements.
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A Cellular Homolog of Hepatitis Delta Antigen: Implications for Viral Replication and Evolution

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Hepatitis delta virus (HDV) is a pathogenic human virus whose RNA genome and replication cycle resemble those of plant viroids. However, viroid genomes contain no open reading frames, whereas HDV RNA encodes a single protein, hepatitis delta antigen (HDAg), which is required for viral replication. A cellular gene whose product interacts with HDAg has now been identified, and this interaction was found to affect viral genomic replication in intact cells. DNA sequence analysis revealed that this protein, termed delta-interacting protein A (DIPA), is a cellular homolog of HDAg. These observations demonstrate that a host gene product can modulate HDV replication and suggest that HDV may have evolved from a primitive viroidlike RNA through capture of a cellular transcript.

HDV was originally discovered as a cause of severe liver injury in individuals already infected with hepatitis B virus (HBV) (*1*). The clinical association between these two viruses results from the fact that HDV does not encode an envelope protein; instead, its genome is enveloped by HBV surface glycoproteins during virus assembly (*2*). The HDV genome comprises a 1700-nucleotide (nt) single-stranded circular RNA that is ~70% self-complementary and, as a result, forms a highly base-paired rodlike structure (*3*). This genome encodes only one known viral protein, HDAg, a nuclear phosphoprotein (*4*) capable of specific binding to HDV RNA (*5*). HDAg is absolutely required for HDV genomic replication (*6*), but the mechanism by which it influences RNA synthesis is unknown.

The HDV genome strongly resembles those of viroids, small naked infectious RNA molecules that produce epidemic disease in many varieties of plants (*7*). Like HDV, viroid genomes are covalently closed, single-stranded circular RNAs with extensive self-complementarity. As in HDV, these viroid RNAs form highly base-paired RNA rods. Viroid RNAs possess ribozyme activities that catalyze RNA self-cleavage and self-ligation (*8*), and similar catalytic activities have been detected in HDV RNA (*9*). However, viroid RNAs are much smaller (<400 nt) and have no open reading frames.

In the currently accepted model of HDV replication, which is based on schemes originally proposed for viroids (*10*), the incoming HDV genome serves as a template for rolling circle replication, resulting in the production of complementary multimeric antigenomes. Nascent antigenomes use their intrinsic ribozyme activities to catalyze self-cleavage and self-ligation, producing circular monomeric antigenomic RNAs. Through a similar rolling circle mechanism, these antigenomes then serve as templates for the production of additional HDV genomic RNAs. Although HDAg is required for replication, it has no known polymerase activity, implying that a host polymerase mediates replication. On the basis of the sensitivity of HDV RNA replication to α-amanitin, the responsible host enzyme has been suggested to be RNA polymerase II (*11*).

Because HDV replication requires HDAg, we speculated that this viral protein might interact with one or more cellular proteins (for example, basal or accessory transcription factors) to promote HDV replication. Accordingly, we used the yeast two-hybrid assay to

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