

Into thin air: Physiology and evolution of alpine insects

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Synopsis Numerous physical parameters that influence insect physiology vary substantially with altitude, including temperature, air density, and oxygen partial pressure. Here, we review existing literature and present new empirical data to better characterize the high-altitude environment, and then consider how this environment affects the physiology and evolution of insects. Using weather balloon data from fifty-three sites across the globe, we estimate a mean altitudinal temperature lapse rate of 6.0 °C/km. We also present empirically determined lapse rates for P_{O₂} and air density. The temperature decline with elevation may substantially compromise insect thermoregulation at high altitude. However, heat-transfer models predict that lower air density at elevation reduces convective heat loss of insects by to a surprisingly large degree. This effect combined with behavioral thermoregulation and the availability of buffered microhabitats make the net thermal consequences of high-altitude residence strongly context-specific. The decline in P_{O₂} with elevation may compromise insect development and physiology, but its effects are difficult to predict without simultaneously considering temperature and air density. Flying insects compensate for low air densities with both short-term responses, such as increased stroke amplitude (but not wingbeat frequency), and with long-term developmental and/or evolutionary increases in wing size relative to body size. Finally, in contrast to predictions based on Bergmann's Rule, a literature survey of thirty-six insect species suggests that those living in colder, higher altitudes *do not* tend to have larger body sizes.

Rapid changes in the physical environment can profoundly alter biological communities across altitudinal gradients. For example, mean temperature drops by approximately 24°C from the base of the Sierra Nevadas (200 m) to the peak of Mount Dana (3979 m) in Yosemite National Park, California. The altitudinal temperature change associated with this short horizontally projected distance (97 km) is roughly equivalent to traveling over 4500 km in latitude (Hopkins, 1938). This rapid temperature reduction is likely a primary factor underlying the striking altitudinal changes in plant and animal communities. However, other physical factors such as oxygen, air density, humidity, and solar radiation may also shape high-altitude insect communities, but have been largely ignored. In this review, we discuss the rapid changes in the physical factors that characterize high altitude, and explore how these gradients influence the physiology and evolution of arthropods, and particularly of insects, living at high altitude. Rather than exhaustively review topics for which data are abundant, we primarily present hypotheses for which data are scarce or nonexistent in the hopes of encouraging future research.

The high-altitude environment

Air temperature profoundly influences organismal physiology. It is therefore critical to know the rate of change of air temperature with altitude. Theoretically, dry air cools adiabatically at a rate of 9.88°C/km (MacArthur, 1972). However, air is seldom completely dry; and when moist air rises and cools water condenses, thereby releasing heat. For several temperate and tropical mountains, moist adiabatic lapse rate (the change in temperature with altitude of a saturated parcel of air) is between 6.0 and 6.5°C/km (Körner, 1999); but we could not find a published global assessment of altitudinal temperature lapse rate.

The demand for precise weather prediction has generated a substantial data set which can be used to empirically determine moist adiabatic lapse rates globally. Weather balloons, released twice daily from sites worldwide, collect detailed climate data as they rise (<http://weather.uwyo.edu/upperair/sounding.html>). We analyzed climate data collected by weather balloons released on two different days (summer and winter solstices of 2003) from 53 sites distributed over the globe (Fig. 1).

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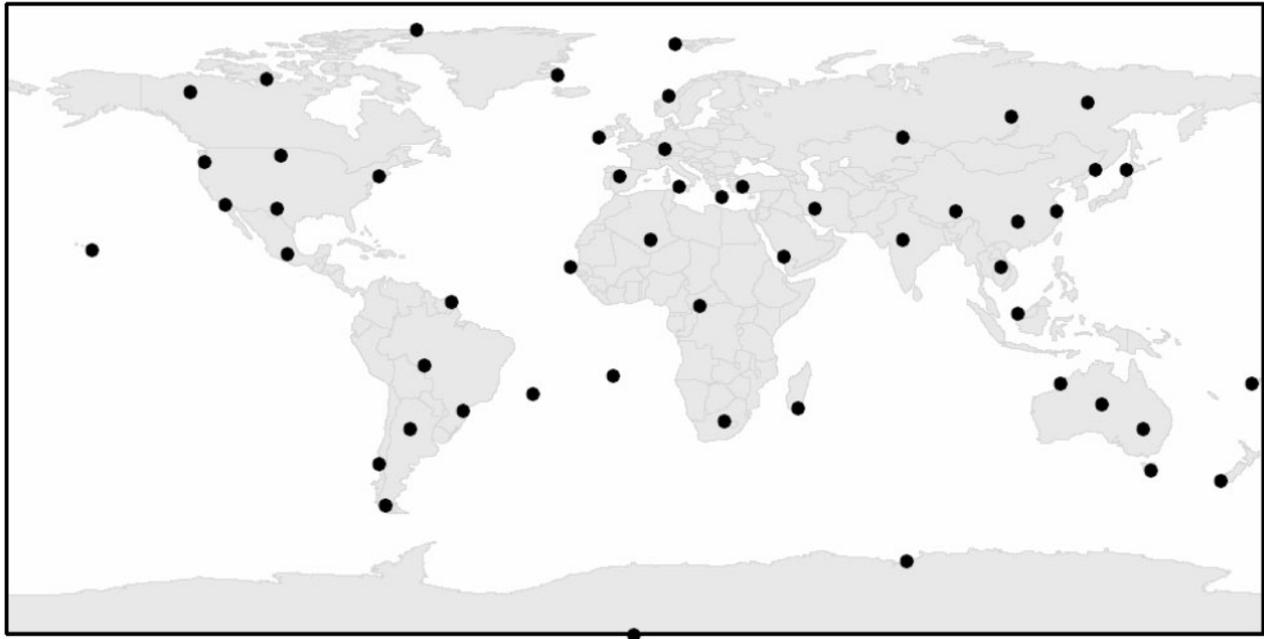


Fig. 1 Locations of 53 weather balloon release sites used in the present analysis. Sites were chosen for their broad spatial distribution and based on availability of data. Map courtesy L. McCoy.

For any given site, air temperature decreased linearly with altitude, as predicted by theory (Fig. 2A). The temperature lapse rate was steeper in summer than in winter (Fig. 2B; paired t-test, $t_{51} = -2.88$, $P = 0.006$; summer mean slope = $-6.2^{\circ}\text{C}/\text{km}$, winter mean slope = $-5.8^{\circ}\text{C}/\text{km}$). Among-site variation in temperature lapse rate was substantial (Fig. 2B) and could not be explained by non-linearity in the data (all linear regressions were strongly supported with $r^2 > 0.60$; see inset of Fig. 2B). Future analyses could determine the extent that factors such as latitude, land-type (continental, coastal, or island), ground temperature and humidity underlie the variation in temperature lapse rate. However, such analyses must first address the multicollinearity among variables and the non-normally distributed data.

A caveat to analyzing weather balloon data is that it may not precisely represent temperature changes along a terrestrial altitudinal gradient, as balloon data are not affected by landscape (NRC Board on Atmospheric Sciences and Climate Committee Panel, 2000; Kalnay and Cai, 2003; Douglass *et al.*, 2004). Local terrain can change wind speed and direction and alter solar heating. Proximity to open water and vegetation can also affect local air temperature. Accordingly, comparisons between temperatures measured by weather stations and weather balloons will be necessary to address this issue.

Weather balloons measure ambient atmospheric pressure in addition to air temperature, allowing us to empirically derive altitudinal lapse rates for oxygen partial pressure (P_{O_2} , estimated as 20.9% of atmospheric pressure; Fig. 2C). P_{O_2} lapse rates were significantly *steeper* in winter than in summer, indicating significant seasonal variation in oxygen availability at high altitudes (paired t-test, $t_{51} = 2.92$, $P = 0.005$; summer: $P_{\text{O}_2} = e^{(3.07-0.131\text{altitude})}$; winter: $P_{\text{O}_2} = e^{(3.07-0.133\text{altitude})}$; Fig. 2D). Although the effects of this variation on humans have been discussed elsewhere (Ward *et al.*, 2000, p. 28; West, 1996), the significance for insects of seasonal variation in P_{O_2} is unexplored.

Weather balloon measurements of temperature, atmospheric pressure, and relative humidity allow us to determine altitudinal variation in air density (Fig. 2E; see Appendix A for equations). Air was denser at low altitudes in the winter relative to summer (significantly different intercepts; paired t-test, $t_{51} = -3.67$, $P = 0.005$; Fig. 2F). However, the density lapse rate was steeper in the winter than in the summer (paired t-test, $t_{51} = 3.42$, $P = 0.001$). Overall, water vapor pressure had a negligible influence on density ($\sim 0.002 \text{ kgm}^{-3}$ averaged across altitude). The reduction in temperature with altitude had large effects on air density. For example, if you neglect to account for the drop in temperature with altitude, your estimate of air density at 6000 m would be equivalent to the true air density at ~ 7500 m.

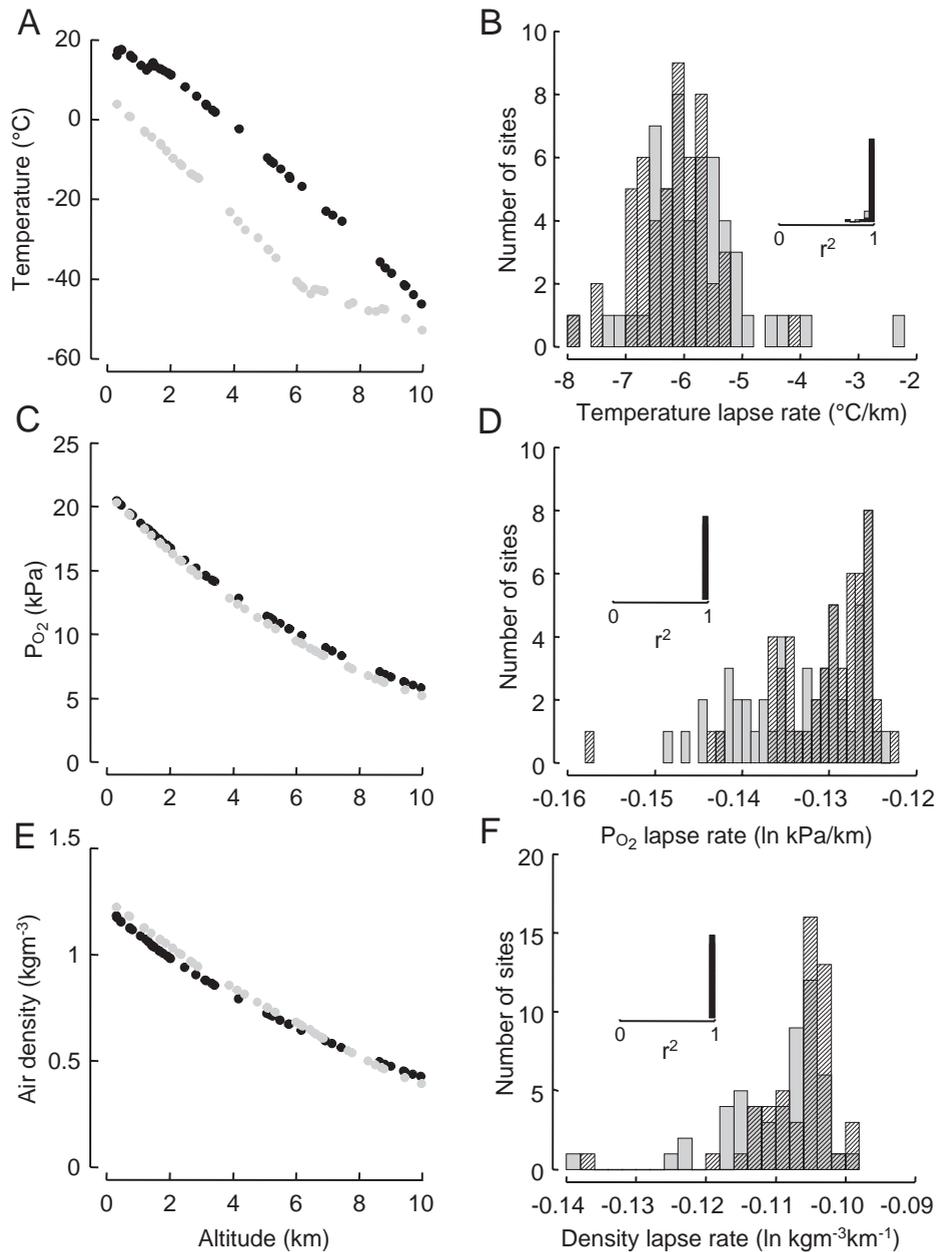


Fig. 2 Air temperature (A), oxygen partial pressure (C), and air density (E) from weather balloons released from Stuttgart, Germany during summer solstice (black circles) and winter solstice (gray circles). Temperature was measured by the weather balloon, P_{O_2} was calculated as 20.9% of the weather balloon pressure measurement, and air density was calculated from weather balloon temperature, pressure, and relative humidity measurements (see Appendix A for equations). Frequency distributions of lapse rates (slopes of regressions relating air temperature, $\ln P_{O_2}$, and \ln air density to altitude) for temperature (B), P_{O_2} (D) and air density (F). Frequency distribution of r^2 values for regressions are given in the inset. Hashed bars indicate summer data and gray bars indicate winter data. Lapse rates of temperature, P_{O_2} , and air density varied significantly with season (see text for details).

Physiological challenges of high altitude

Thermoregulation

The decrease in mean air temperature with altitude does not necessarily result in equivalent changes in arthropod body temperature (Stevenson, 1985). Body

temperature is determined by multiple heat transfer processes that depend on characteristics of the microhabitat as well as organism size, shape, and behavior (Porter and Gates, 1969; Bakken, 1976; Casey, 1992). Microhabitats vary due to factors such as proximity to and thickness of the boundary layer, radiative heating, and even heat from other organisms or structures

(Mani, 1968; Somme, 1989; Walsberg, 1992). Behavioral changes in diurnal and seasonal activity with altitude may also reduce exposure to temperature extremes (Kingsolver, 1983; Somme, 1989; Huey, 1991; Dahlgaard *et al.*, 2001; Ashby, 1997). These factors, and the relatively small size of arthropods, make it difficult to assess temperatures experienced in the field. However, Jones *et al.* (1987) used a novel technique to determine developmental temperatures of *Drosophila melanogaster* across an altitudinal gradient (40–1000 m). They released at different altitudes mutant flies for which adult eye color was determined by ambient temperatures experienced by the pupae. The eye color of adult flies collected from the field revealed the temperature experienced during development. Although mean air temperature dropped by 4°C from the low- to high-elevation site, mean developmental temperature of the flies was reduced by only 1°C. Similarly, grasshoppers (*Xanthippus corallipes*) collected at 3500 m maintained body temperatures only 7.8°C less than grasshoppers living at 1600 m despite a 10.6°C difference in air temperature and a 17.0°C difference in ground temperature between these sites (Ashby, 1997). These studies highlight the importance of behavior and microclimate in buffering the dramatic altitudinal drop in temperature (see also Huey, 1991).

Many climatic factors other than temperature have been extensively studied and shown to influence body temperature of arthropods (reviewed by Mani, 1968; Sømme, 1989). For example, at higher altitudes, drier air may augment evaporative heat loss whereas reduced atmospheric scattering of incident radiation may increase solar heat load. One factor that has received little attention, however, is the effect of reduced air density on convective heat transfer (but see Huey *et al.*, 2001).

Convection is often the dominant mode of heat transfer for small arthropods and is estimated by the following equation (Monteith and Unsworth, 1990; Porter and Gates, 1969; Casey, 1992):

$$Q_c = h_c A (T_b - T_e) \quad (1)$$

where Q_c is convective heat loss, h_c is the convective heat transfer coefficient, A is surface area, and T_b and T_e are temperatures of the body and environment, respectively. It is well documented that convective heat loss is affected by environmental temperature and body size (influencing $T_b - T_e$ and A , respectively; Stevenson, 1985). However, the effects of altitude *per se* on h_c for insects remain unexplored.

To calculate h_c under equilibrium conditions, we model insects as geometrically similar cylinders and use the empirically derived dependence of the

Nusselt number (Nu) on the Reynolds number (Re) for a cylinder in the correct Re range (40–4000; Monteith and Unsworth, 1990):

$$Nu = 0.62 Re^{0.47} \quad (2)$$

Nu describes the ratio of convective to conductive heat transfer and is given by:

$$Nu = \frac{h_c L}{k} \quad (3)$$

Re is a non-dimensional number indicating the ratio of convective to viscous transport of momentum in the fluid and is given by:

$$Re = \frac{\rho U L}{\mu} \quad (4)$$

where L is a characteristic length (calculated as $mass^{1/3}$), k is the thermal conductivity of the fluid, ρ is the fluid density, U is the fluid velocity, and μ is the dynamic viscosity of the fluid (Denny, 1993; Monteith and Unsworth, 1990; Campbell, 1977). Altitudinal variation in air density (ρ ; Fig. 2E, F), thermal conductivity (k), and dynamic viscosity (μ) all potentially alter h_c and therefore convective heat exchange (Q_c). We therefore incorporated altitudinal variation in these properties of air (US Standard Atmosphere, 1976) into an analysis of convective heat loss (equations 1–4) for an idealized homeothermic insect (approximated by a cylinder) with body temperature (T_b) regulated at 40°C either by endothermic heat production (e.g. bumblebees and moths; Heinrich, 1993) or by behavioral thermoregulation (e.g. grasshoppers: Ashby, 1993; butterflies: Kingsolver, 1983). For this analysis, we used a sea-level air temperature (T_e) of 25°C, a temperature lapse rate of 6°C/km (Fig. 2B) and two wind speeds (1 and 6 m/s).

Reduced air density significantly reduces convective heat loss at altitude, especially at high wind speeds (Fig. 3). At 6000 m, actual convective heat loss is 26% less than it would be for a constant density atmosphere (Fig. 3). This difference is equivalent thermally to increasing ambient air temperature at 6000 m by 10°C (eq. 1). Altitudinal variation in thermal conductivity and in dynamic viscosity have smaller effects on convective heat transfer (unpublished data, M.E.D.). Although this model estimates heat loss for large homeothermic insects, the general result (that convective heat transfer is reduced at high altitude) holds for any insect. The altitudinal reduction in convective heat transfer will make it easier for insects to warm up and stay hot and more difficult for them to cool down. However, a full biophysical analysis that includes altitude has yet to be done for any organism. As such, we don't know the potential implications of this phenomenon for insect thermoregulation at high altitude.

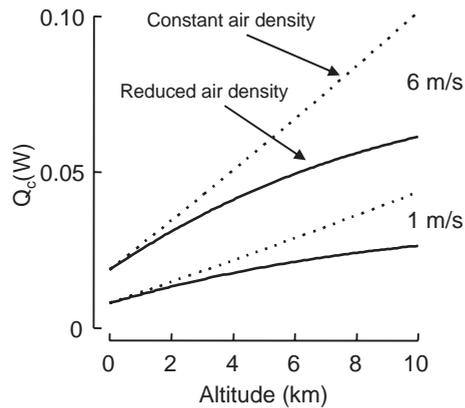


Fig. 3 Instantaneous convective heat loss across altitude for an idealized homeothermic insect (approximated by a cylinder) at two wind speeds assuming an initial body temperature of 40°C, a sea level air temperature of 25°C and a 6°C/km decrease in air temperature with altitude. See text for equations and details. Solid lines represent the estimate of true convective heat loss taking into account the empirical decrease in air density with altitude; dotted lines indicate convective heat loss predicted for a constant-density atmosphere.

Hypoxia

Unlike operative temperature, which insects may manipulate to varying degrees, the partial pressure of atmospheric oxygen is beyond an insect's control. Low P_{O_2} can acutely affect human performance, but it is unclear how it affects the physiological and life history traits of insects at high altitude.

Many studies have shown that *short-term* exposure to ecologically realistic P_{O_2} levels has little or no effect on insect metabolic rate or survival (reviewed by Hoback and Stanley, 2001; Greenlee and Harrison, 2004). However, at high altitude, insects experience constant low oxygen. Chronic exposure of hawkmoth (*Manduca sexta*) eggs to low but ecologically realistic P_{O_2} causes reduced metabolic rates, longer hatching times, and greater mortality (Woods and Hill, 2004). Similarly, chronic exposure to low P_{O_2} slows development, reduces survival, and results in smaller body size in both mealworms (*Tenebrio molitor*; Loudon, 1988; Greenberg and Ar, 1996) and fruitflies (*Drosophila melanogaster*; Frazier *et al.*, 2001).

The effects of chronic exposure to low P_{O_2} should be studied in conjunction with temperature, because temperature dramatically alters the physiological effects of reduced P_{O_2} on ectotherms (Sibley and Atkinson, 1994; Atkinson, 1996; Woods 1999; Portner, 2001). High temperatures exacerbate the effects of low P_{O_2} because, in ectotherms, the metabolic demand for oxygen increases exponentially with temperature (Gillooly *et al.* 2001) while oxygen diffusion rates increase only modestly (about 5% per 10°C; Denny, 1993). Several

studies support the prediction that insects have more difficulty meeting tissue oxygen demands at warmer temperatures. For hawkmoth eggs, metabolic rate, survival, and hatching time were most affected by low P_{O_2} at high temperatures; in fact, at the highest rearing temperatures (32–37°C), sea level P_{O_2} proved limiting (Woods and Hill, 2004). *Drosophila melanogaster* reared in hypoxia and cool temperatures were 10–15% smaller than those reared in sea level P_{O_2} , whereas flies reared in hypoxia and warm temperatures were 30–40% smaller (Frazier *et al.*, 2001). Growth rate and development time of this species showed similar trends. Evidence of interactive effects between temperature and oxygen delivery are also observed in other ectothermic organisms (Keister and Buck, 1961; Bryan *et al.*, 1984; Hicks and Wood, 1985; Dupre and Wood, 1988; Schurmann and Steffensen, 1992; Donahaye *et al.*, 1996; Frederick and Portner, 2000).

To understand the effects of low P_{O_2} on alpine insects, we need to better characterize both the chronic effects of low P_{O_2} and insect body temperatures along altitudinal gradients. One prediction is that the effects of low P_{O_2} at altitude are mitigated by the concurrent decline in mean air temperature (Fig. 2A). However, endothermic insects that maintain high body temperatures, such as many bees, large flies, and moths may be particularly affected by low P_{O_2} . Ectothermic insects may also be strongly affected by low P_{O_2} at altitude because mean air temperature often does not represent the body temperature of ectothermic organisms (see “Thermoregulation” section). In addition, maximum air temperatures at high altitudes can meet or exceed those of lower altitudes; and microhabitats can be prohibitively hot, even at high altitudes.

It is important to note that in aforementioned studies, partial pressure of oxygen was manipulated by reducing the percentage of oxygen using nitrogen replacement within a normobaric gas mixture; air density thus remained close to the sea-level value. However, increasing altitude causes an absolute reduction in air density as well as in partial pressure of oxygen. Although reduced oxygen partial pressure decreases the driving force for oxygen diffusion according to Fick's Law, the concomitant reduction in air density theoretically lowers the medium's resistance to gaseous diffusion (*i.e.*, larger diffusion coefficient) exactly compensating for the reduction in partial pressure (Denny, 1993). For insects in which air-based diffusion is the limiting step of oxygen delivery, the high-altitude environment may not compromise tissue oxygen delivery even if there is no physiological compensation for reduced P_{O_2} . The combined effects of temperature, oxygen, and air density on respiration and development are still unstudied. Factorial experiments

that decouple temperature, oxygen, and air density using variable-density gas mixtures (see Dudley and Chai, 1996) would allow for measurement of both the direct and interactive effects of these factors on insect development.

Respiratory responses to low P_{O_2}

If reduced P_{O_2} at high altitude challenges tissue oxygen delivery, insects may adaptively respond by altering the respiratory system. The insect respiratory system delivers oxygen to tissues via a network of highly branched tubes (tracheae) that terminate in body tissues (Wigglesworth, 1972; Ghabrial *et al.*, 2003). To compensate for low oxygen, insects increase both the number of terminal tracheal branches (Wigglesworth, 1954; Jarecki *et al.*, 1999) and the diameter of primary tracheae (Locke, 1958; Loudon, 1989; Henry and Harrison, 2004). In larval *D. melanogaster*, tracheolar density responds to tissue oxygen needs with remarkable precision and rapidity. Cells experiencing hypoxia release a local molecular signal, Branchless (Bnl), that induces sprouting in nearby terminal tracheal branches and directs them to the tissue (Jarecki *et al.*, 1999). Using the GAL4-UAS reporter system (Brand and Perrimon, 1993), Jarecki *et al.* (1999) demonstrated that extensive proliferation of terminal tracheal branches can occur within one hour. Reduced oxygen also triggers a developmental increase in the diameter of primary tracheae in mealworms (Locke, 1958; Loudon, 1989) and in fruit flies (Henry and Harrison, 2004), as well as heritable increases in primary trachea size in *D. melanogaster* following several generations in low oxygen (Henry and Harrison, 2004).

Given the developmental and evolutionary plasticity of the insect tracheal system, high altitude environments may not typically compromise oxygen delivery. However, if thorax volume remains constant, increased tracheal volume will reduce the body volume available for other structures such as flight muscle or ovaries. Among species, differences in the relative allocation of muscle fibers, mitochondria, tracheae, and sarcoplasmic reticulum within a limited thoracic volume result in significant trade-offs in flight performance (Conley and Lindstedt, 2002). If high-altitude insects replace muscle, mitochondria, or sarcoplasmic reticulum with trachea or air sacs, their flight performance, which is already challenged at high altitudes (see below), may be further compromised.

Flight physiology

Insects should have reduced flight performance at altitude because of reduced mean air temperatures and changes in the physical properties of air. Low

temperatures can compromise flight by altering metabolism and muscle physiology (Scaraffia and Burgos 2000; Josephson, 1981; Hosler *et al.*, 2000). Reduced air density at high altitude reduces both Re and aerodynamic forces generated by insect wings (Vogel, 1994; Dudley, 2000). High altitude may therefore compromise flight because a greater downward flux of air is required to maintain the constant momentum flux necessary to offset body weight (air viscosity also determines forces but varies little with altitude; see Dudley and Chai, 1996). The power required to produce this momentum flux, the induced power, therefore also increases at high altitude (Dudley, 2000).

Despite the aerodynamic challenges, insects in the laboratory and on mountains are able to fly in reduced density air. Mosquitoes fly at air densities only 20% of the normobaric value (Galun and Fraenkel, 1961). Orchid bees (Apidae: Euglossini) in normoxic but hypodense gas mixtures can hover at air densities 36% of the sea-level value (Dudley, 1995), and honeybees fly in hypobaric air corresponding to about 4500 m, or 65% of normobaric density (Withers, 1981). To fly in these low air densities, these insects must be changing the three-dimensional motions of their wings (*i.e.*, the wingbeat kinematics). Kinematic data for insects flying in hypodense air are available only for orchid bees and bumblebees in the laboratory; the primary means of compensation is an increase in stroke amplitude (the angular extent of wing motion; see Dudley, 2000), whereas wingbeat frequency remains constant (Dudley, 1995; unpublished data, M.E.D.). Orchid bees exhibit a similar response during maximum load-lifting tests, albeit in normobaria (Dillon and Dudley, 2004). Whether insects flying at high altitudes exhibit similar changes in wingbeat kinematics remains untested.

On a developmental or evolutionary timescale, flying insects may compensate for reduced air density by altering wing or body morphology. Insects with longer wings relative to body size reduce loss of momentum from the tip of the wing, decreasing induced power requirements of flight (Dudley, 2000). Insects with greater wing area relative to body size decrease the induced velocity necessary to sustain flight, thereby reducing induced power expenditure (Dudley, 2000). High-altitude insects may therefore minimize induced power by changing wing length and/or wing area relative to body size. Some morphological data support this hypothesis. Wing length of *Drosophila robusta* is greater at higher elevations whereas thoracic dimensions remain constant (Stalker and Carson, 1948). Similarly, mountain honeybees have longer wings and greater wing area but invariant body mass

relative to their lowland counterparts (Hepburn *et al.*, 1998).

We further tested this hypothesis with bumblebees which are prominent alpine pollinators. We studied museum specimens of *Bombus festivus*, a species found across a wide altitudinal range (400 to 5200 m) and present in large numbers in the collections of the Institute of Zoology, Beijing, and the National Museum of Natural History, Washington D.C. Wet mass was not available for museum bumblebees, so we instead measured the width of the thorax at the lateral margins of the wing bases (the interalar width). We also measured the length of a flattened forewing for each specimen. For *Bombus festivus* queens, wing length increases with altitude (ANOVA, $F_{1,236} = 9.89$, $P = 0.002$; Fig. 4A, open circles), but wing length relative to a reliable proxy for body mass (*i.e.*, the cube of interalar width; based on unpublished data, M.E.D. and the standard scaling relationship that $\text{mass} \sim \text{length}^{1/3}$) does not change (ANOVA, $F_{1,236} = 0.364$, $P = 0.567$). For workers of this species, wing length decreases with altitude (ANOVA, $F_{1,60} = 4.06$, $P = 0.048$; Fig. 4B, open circles), but wing length relative to our proxy for body mass increases with altitude (ANOVA, $F_{1,60} = 4.15$, $P = 0.048$). For male *B. festivus*, neither wing length (ANOVA, $F_{1,33} = 2.00$, $P = 0.167$; Fig. 4C, open circles) nor wing length relative to our proxy for body mass (ANOVA, $F_{1,33} = 0.683$, $P = 0.415$) changes significantly with altitude. For this bee species, only the flight morphology of workers changes to help offset the increased induced power requirements of flight at high altitude. It is tempting to speculate that this morphological difference is related to caste differences in foraging behavior (only workers carry loads throughout their lifespan), but more data are necessary to test this hypothesis.

Altitudinal changes in wingbeat kinematics and morphology have important consequences for flight performance. In hummingbirds, systematic inter-specific variation in morphology and kinematics with elevation is accompanied by a systematic decline in the power margin (the ratio of maximum power when load-lifting relative to unloaded hovering; Altshuler and Dudley, 2003; Altshuler *et al.*, 2004). Hummingbirds at higher elevations operate closer to their maximum power availability during normal hovering, leaving less room for supplemental modulation of force and power production during escapes or sexual displays. A similar result might be expected for insects across elevational gradients, but this hypothesis has not been tested. Of particular interest for insects is whether low oxygen availability interacts with reduced air density to limit flight. Also, increased

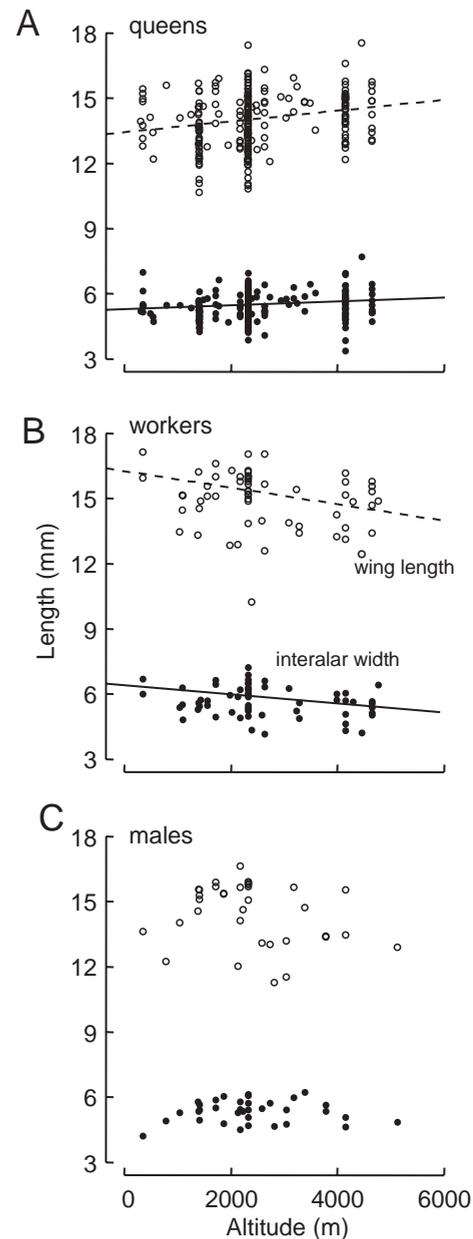


Fig. 4 Altitudinal variation in interalar width (IW; filled circles) and wing length (WL; open circles) for *Bombus festivus*: (A) queens, (B) workers, and (C) males. Only significant regression lines are shown. Queens ($n = 238$): $\text{IW} = 5.3 + 8.7e-5\text{altitude}$ ($P = 0.03$, $r^2 = 0.02$); $\text{WL} = 13.4 + 2.5e-4\text{altitude}$ ($P = 0.002$, $r^2 = 0.04$). Workers ($n = 61$): $\text{IW} = 6.4 - 2.0e-4\text{altitude}$ ($P = 0.03$, $r^2 = 0.08$); $\text{WL} = 16.3 - 4.0e-4\text{altitude}$ ($P = 0.04$, $r^2 = 0.06$). Males ($n = 34$): IW ($P = 0.91$); WL ($P = 0.17$).

oxygen demand, which may characterize flight at high altitudes, can increase water loss in insects (Lehmann, 2001). Future experimental manipulations might include total density reduction under both normoxic and hyperoxic conditions to determine whether increased oxygen availability augments maximum performance. In particular, effects of air density, oxygen

partial pressure, and air temperature can either be experimentally decoupled or analyzed in concert (Dudley and Chai, 1996), thus allowing biomechanical effects of reduced air density on flight aerodynamics to be identified independent of aerobic constraints on flight.

Patterns along altitudinal gradients

Intraspecific body size clines

The observation that individuals of many species are larger at higher latitudes has long intrigued biologists (Chown and Klok, 2003; Angilletta and Sears, 2004; Blanckenhorn and Demont, 2004). Because latitudinal clines in body size are developmentally (Atkinson, 1994) and evolutionarily (Partridge *et al.*, 1994) linked with temperature, biologists have also searched for body size variation along elevational gradients. Specifically, the question we address is: for a given insect species are populations at colder high-altitude sites larger than those living at warmer low-altitude sites? We use two approaches to answer this question. First we evaluate in detail the available data for a bumblebee species (Hymenoptera: Apidae, genus *Bombus*) which occurs across a wide altitudinal gradient. Second, we summarize existing data on body size clines of other insects across elevational gradients.

Our analysis of *Bombus festivus* body size clines presents a mixed pattern: queens of this species are larger at higher altitude (Fig. 4A, filled circles; ANOVA, $F_{1,237} = 4.86$, $P = 0.03$); workers are smaller (Fig. 4B, filled circles; ANOVA, $F_{1,60} = 4.90$, $P = 0.03$); and males show no significant change in body size (Fig. 4C, filled circles; ANOVA, $F_{1,33} = 0.01$, $P = 0.91$). These among-caste differences in body size clines may reflect both behavioral and developmental differences.

We also reviewed published studies of intraspecific variation in body size with altitude deriving both from phenotypic plasticity during development and from adaptation across elevational gradients. The prediction based on latitudinal clines, that insects will be larger at colder high-altitude sites, is clearly contradicted. Among twenty-nine species from four insect orders, we did not observe increased body size clines more often than predicted by chance ($\chi^2_{2,25} = 2.6$, $P = 0.27$; Table 1: field-caught). Similar results were obtained for a second analysis that excluded nine species that showed no significant body size cline, because this result could be due to either a lack of statistical power or a true lack of an altitudinal body size cline (field-caught: $\chi^2_{1,17} = 0.6$, $P = 0.44$). Evolutionary forces may select for genetically larger insects at high altitude, but body size may still be reduced during development because of resource limitation. In a few

studies, insects from different elevations were reared in common conditions to distinguish between phenotypic plasticity and adaptive differences in body size along elevational gradients. The data for these six species do not support the prediction that larger body size has evolved in high-altitude insects ($\chi^2_{2,2} = 0.4$, $P = 0.82$; data with no significant body size trend removed: $\chi^2_{1,1} = 0.33$, $P = 0.56$; Table 1: laboratory-reared), but the sample size is limited. This should be regarded as a preliminary analysis because these species do not represent independent samples, and should be analyzed with methods that control for phylogenetic history.

Several hypotheses may explain why altitudinal trends in body size do not mirror those observed along latitudinal gradients. Shorter geographic distances across altitudinal gradients may lead to high gene flow, limiting the possibility for development of a cline (Garcia-Ramos and Kirkpatrick, 1997; Kirkpatrick and Barton, 1997; Doebeli and Dieckmann, 2003). Also, altitudinal gradients include a systematic reduction in P_{O_2} which can result in smaller body size. An alternative hypothesis is that altitudinal and latitudinal gradients actually *are* functionally equivalent, and the apparently positive correlation between *latitude* and body size in ectothermic organisms is spurious. Although many studies have shown intraspecific increases in insect body size along latitudinal gradients, others have found opposite trends (reviewed by Chown and Gaston, 1999; Blanckenhorn and Demont, 2004).

Biodiversity

Given the pronounced changes in temperature, air density, and oxygen availability with elevation, as well as their direct physiological consequences, what are the broader implications for arthropod diversity? As with most metazoan taxa, species richness (*i.e.*, the absolute # of species) and species density (# species per unit habitat area) tend to peak at intermediate elevations, typically between 1000 and 2000 meters (Janzen, 1973; Janzen *et al.*, 1976; Rahbek, 1995; Lomolino, 2001; Sanders, 2002). Diverse factors have been implicated in such patterns, including variation in available habitat area at different elevational bands, changes in plant primary productivity, climatic limitations, and the source effect of lowland species contributing to mid-elevation faunas (Lawton *et al.*, 1987; McCoy, 1990; Sanders, 2002).

Overall, faunistic changes with elevation typically parallel those seen in latitudinal species gradients, with reduced diversity at climatic extremes. One general ecological effect influencing both tropical

Table 1 Insect body size clines along altitudinal gradients from published studies for species caught in the field or reared in a common laboratory environment

| Species | Size cline* | Body part** | Reference |
|--|-------------|-----------------|--------------------------------|
| Field-caught | | | |
| Coleoptera | | | |
| <i>Adesmia metallica</i> | – | elytral l. | Krasnov <i>et al.</i> , 1996 |
| <i>Agonum assimile</i> | – | NA | Thiele and Kirchner, 1958*** |
| <i>Anatolica paphia</i> | – | body l. | Mani, 1968 |
| <i>Blaps caraboides</i> | – | body l. | Mani, 1968 |
| <i>Bothrometopus brevis</i> | 0 | head l. | Chown and Klok, 2003 |
| <i>Bothrometopus elongatus</i> | + | head l. | Chown and Klok, 2003 |
| <i>Bothrometopus gracilipes</i> | – | head l. | Chown and Klok, 2003 |
| <i>Bothrometopus parvulus</i> | + | head l. | Chown and Klok, 2003 |
| <i>Canonopsis sericeus</i> | – | head l. | Chown and Klok, 2003 |
| <i>Ectemnorhinus marioni</i> | + | head l. | Chown and Klok, 2003 |
| <i>Ectemnorhinus similes</i> | + | head l. | Chown and Klok, 2003 |
| <i>Ectemnorhinus viridis</i> | – | head l. | Chown and Klok, 2003 |
| <i>Erodium edomitus</i> | – | elytral l. | Krasnov <i>et al.</i> , 1996 |
| <i>Micipsa philistina</i> | 0 | elytral l. | Krasnov <i>et al.</i> , 1996 |
| <i>Pimelia canescens</i> | 0 | elytral l. | Krasnov <i>et al.</i> , 1996 |
| <i>Platyscelis margellanica</i> | – | body l. | Mani, 1968 |
| <i>Prosodes alaiensis</i> | – | body l. | Mani, 1968 |
| <i>Prosodes costifera</i> | – | body l. | Mani, 1968 |
| <i>Pterostichus vulgaris</i> | – | NA | Thiele and Kirchner, 1958*** |
| <i>Sepidium dathan</i> | + | elytral l. (f) | Krasnov <i>et al.</i> , 1996 |
| <i>Trachyderma philistina</i> | 0 | elytral l. | Krasnov <i>et al.</i> , 1996 |
| <i>Zophosis complanata</i> | – | elytral l. | Krasnov <i>et al.</i> , 1996 |
| Diptera | | | |
| <i>Drosophila buzzatii</i> | 0 | thorax l. (f) | Dahlgaard <i>et al.</i> , 2001 |
| <i>Drosophila mediopunctata</i> | 0 | (f) | Bitner-Mathe and Klaczko, 1999 |
| <i>Drosophila robusta</i> | 0 | thorax l. (f) | Stalker and Carson, 1948 |
| <i>Scathophaga stercoraria</i> | 0 | hind tibia l. | Blanckenhorn, 1997 |
| <i>Sepsis cynipsea</i> | 0 | head w. | Blanckenhorn, 1997 |
| Neuroptera | | | |
| <i>Myrmeleon immaculatus</i> | – | intereye w. (f) | Arnett and Gotelli, 1999 |
| <i>Myrmeleon immaculatus</i> | 0 | intereye w. (m) | Arnett and Gotelli, 1999 |
| Orthoptera | | | |
| <i>Hemideina maori</i> | + | head l. | Koning and Jamieson, 2001. |
| Field-caught totals: + (6); - (13); 0 (9); mixed results (1; <i>M. immaculatus</i>) | | | |
| Lab-reared | | | |
| Diptera | | | |
| <i>Drosophila buzzatii</i> | – | thorax l. | Norry <i>et al.</i> , 2001 |
| <i>Drosophila buzzatii</i> | + | thorax l. (f) | Dahlgaard <i>et al.</i> , 2001 |
| <i>Drosophila buzzatii</i> | + | thorax l. (f) | Bubliy and Loeschcke, 2005 |
| <i>Drosophila mediopunctata</i> | + | PC analysis (f) | Bitner-Mathe and Klaczko, 1999 |
| <i>Drosophila simulans</i> | 0 | thorax l. (f) | Bubliy and Loeschcke, 2005 |
| <i>Scathophaga stercoraria</i> | – | hind tibia l. | Blanckenhorn, 1997 |
| <i>Sepsis cynipsea</i> | 0 | head w. | Blanckenhorn, 1997 |
| <i>Zaprionus indianus</i> | + | thorax l. (f) | Karan <i>et al.</i> , 2000 |
| Lab-reared totals: + (2); - (1); 0 (2); mixed results (1; <i>D. buzzatii</i>) | | | |

*Indicates whether body size significantly increased with altitude (+; i.e. Bergmann's Rule), showed no significant change with altitude (0), or significantly decreased (–) with altitude.

**Measurement used to estimate body size (references using wings were excluded because wings and body size can vary independently in response to rearing conditions). l. = length, w. = width, m = male data, f = female data, when gender is not indicated males and females had the same trend, were pooled, or not differentiated.

***Cited in Sømme, 1989

and mid-elevational diversity may be the mid-domain effect (Colwell, 2000), whereby an upper limit to species range reduces richness at the ends of a geographical distribution, and correspondingly enhances diversity towards the geographical center. For insects across altitudinal gradients, tests of the mid-domain hypothesis are scarce and present mixed results. The mid-domain effect explains very little of variation in species diversity of Nevada butterflies (Fleishmann *et al.*, 1998). For Madagascar butterflies, the mid-domain effect explains as much as 75% of an altitudinal diversity gradient, but underestimates species diversity at low elevations and predicts a peak in species diversity at a lower altitude than the empirically observed peak (Lees *et al.*, 1999). For ants in Colorado, Nevada, and Utah, the strength of the mid-domain effect on species diversity varies considerably with state (13–90% of the variation in species diversity explained), but in all states it predicts a peak in species diversity higher than the observed peak (Sanders, 2002). More studies are clearly needed to determine the importance of geometrical constraints (*i.e.*, habitat area, mid-domain effect) in determining altitudinal gradients in species diversity.

Many features of insect altitudinal distributions may derive from correlated variation in floristic composition and associated nutritional resources. Such effects may be particularly pronounced in flower-pollinator mutualisms. Lower air temperatures and increased aerodynamic costs of flight with elevation must impose increased energetic costs on volant insects (although this has not been explicitly tested), for which flowers in turn might provide greater nectar rewards (Heinrich, 1975). Surveys of floral nectar production across elevational gradients (*e.g.*, Cruden *et al.*, 1983) yield no systematic pattern, and no published data exist on sugar concentrations and overall nectar availability for those flowers actually visited by pollinating insects at different elevations. However, behavioral adaptations and taxonomic specialization among suites of pollinators clearly enable the maintenance of effective pollination regimes at high elevation (*e.g.*, Arroyo *et al.*, 1982; Bingham and Orthner, 1998). Potential linkage between mechanistic constraints on insect behavior and performance and ecological interactions remains an interesting prospect for future studies of alpine pollination systems.

Appendix A

Density (ρ) is given by:

$$\rho = \frac{P_{dry}}{287.1T} + \frac{P_{water}}{461.5T}$$

where T is absolute temperature (K), and the pressure exerted by all air components other than water (P_{dry} , Pa)

was calculated from the relationship between the weather balloon pressure measurement (P_{total} , Pa) and the local water vapor pressure (P_{water} , Pa):

$$P_{total} = P_{dry} + P_{water}$$

P_{water} was estimated from the weather balloon relative humidity measurement (h) and the saturation vapor pressure (e_s , Pa):

$$P_{water} = \frac{he_s}{100}$$

Over the temperature range of -40 to 50°C , e_s (mb) is dependent on temperature (T , C) and pressure (P_{total} mb) as follows (Buck 1981):

$$e_s = (1.007 + 3.46E^{-6}P)6.1121e^{\frac{17.502T}{240.97+T}}$$

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References

- Altshuler, D. L. and R. Dudley. 2003. Kinematics of hovering hummingbird flight along simulated and natural elevational gradients. *J. Exp. Biol.* **206**:3139–3147.
- Altshuler, D. L., R. Dudley, and J. A. McGuire. 2004. Resolution of a paradox: Hummingbird flight at high elevation does not come without a cost. *Proc. Natl. Acad. Sci. U.S.A.* **101**:17731–17736.
- Angilletta, M. J. and M. W. Sears. 2004. Evolution of thermal reaction norms for growth rate and body size in ectotherms: An introduction to the symposium. *Integr. Comp. Biol.* **44**:401–402.
- Arnett, A. and N. Gotelli. 1999. Bergmann's rule in the ant lion *Myrmeleon immaculatus* DeGeer (Neuroptera : Myrmeleontidae): geographic variation in body size and heterozygosity. *J. Biogeogr.* **26**:275–283.
- Arroyo, M. T. K., R. Primack, and J. Armesto. 1982. Community structure in pollination ecology in the high temperate Andes of Central Chile I. Pollination mechanisms and altitudinal variation. *Am. J. Bot.* **69**:82–97.

- Ashby, P. D. 1997. Conservation of mass-specific metabolic rate among high- and low-elevation populations of the acridid grasshopper *Xanthippus corallipes*. *Physiol. Zool.* **70**:701–711.
- Atkinson, D. 1994. Temperature and organism size—a biological rule for ectotherms. *Adv. Ecol. Res.* **25**:1–58.
- Atkinson, D. 1996. On the solutions to a major life-history puzzle. *Oikos* **77**:359–365.
- Bakken, G. S. 1976. A heat transfer analysis of animals: Unifying concepts and the application of metabolism chamber data to field ecology. *J. Theor. Biol.* **60**:337–384.
- Bingham, R. A. and A. R. Orthner. 1998. Efficient pollination of alpine plants. *Nature* **391**:238–239.
- Bitner-Mathe, B. C. and L. B. Klaczko. 1999. Size and shape heritability in natural populations of *Drosophila mediopunctata*: Temporal and microgeographical variation. *Genetica* **105**:35–42.
- Blanckenhorn, W. U. 1997. Altitudinal life history variation in the dung flies *Scathophaga stercoraria* and *Sepsis cynipsea*. *Oecologia* **109**:342–352.
- Blanckenhorn, W. U. and M. Demont. 2004. Bergmann and converse Bergmann latitudinal clines in arthropods: Two ends of a continuum? *Integr. Comp. Biol.* **44**:413–424.
- Brand, A. H. and N. Perrimon. 1993. Targeted gene-expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**:401–415.
- Bryan, J. D., L. G. Hill, and W. H. Neill. 1984. Interdependence of acute temperature preference and respiration in the plains minnow. *Trans. Am. Fish. Soc.* **113**:557–562.
- Bubliy, O. A. and V. Loeschcke. 2005. Variation of life-history and morphometrical traits in *Drosophila buzzatii* and *Drosophila simulans* collected along an altitudinal gradient from a Canary island. *Biol. J. Linn. Soc.* **84**:119–136.
- Buck, A. L. 1981. New equations for computing vapor pressure and enhancement factor. *J. Appl. Meteorol.* **20**:1527–1532.
- Campbell, G. S. 1977. *An introduction to environmental biophysics*. Springer-Verlag, New York.
- Casey, T. M. 1992. Biophysical ecology and heat-exchange in insects. *Amer. Zool.* **32**:225–237.
- Chown, S. L. and K. J. Gaston. 1999. Exploring links between physiology and ecology at macro-scales: The role of respiratory metabolism in insects. *Biol. Rev. Camb. Philos. Soc.* **74**:87–120.
- Chown, S. L. and C. J. Klok. 2003. Altitudinal body size clines: Latitudinal effects associated with changing seasonality. *Ecography* **26**:445–455.
- Colwell, R. K. 2000. The mid-domain effect: Geometric constraints on the geography of species richness. *Trends Ecol. Evol.* **15**:70–76.
- Conley, K. E. and S. L. Lindstedt. 2002. Energy-saving mechanisms in muscle: The minimization strategy. *J. Exp. Biol.* **205**:2175–2181.
- Cruden, R. W., S. M. Hermann, and S. Peterson. 1983. Patterns of nectar production and plant-pollinator coevolution. In B. Bentley and T. Elias (eds), *The biology of nectaries*, pp. 80–125. Columbia University Press, New York.
- Dahlgard, J., E. Hasson, and V. Loeschcke. 2001. Behavioral differentiation in oviposition activity in *Drosophila buzzatii* from highland and lowland populations in Argentina: Plasticity or thermal adaptation? *Evolution* **55**:738–747.
- Denny, M. W. 1993. *Air and water: The biology and physics of life's media*. Princeton University Press, New Jersey.
- Dillon, M. E. and R. Dudley. 2004. Allometry of maximum vertical force production during hovering flight of neotropical orchid bees (Apidae: Euglossini). *J. Exp. Biol.* **207**:417–425.
- Doebeli, M. and U. Dieckmann. 2003. Speciation along environmental gradients. *Nature* **421**:259–264.
- Donahaye, E., S. Navarro, M. Rindner, and A. Azrieli. 1996. The combined influence of temperature and modified atmospheres on *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *J. Stored Prod. Res.* **32**:225–232.
- Douglass, D. H., B. D. Pearson, S. F. Singer, P. C. Knappenberger, and P. J. Michaels. 2004. Disparity of tropospheric and surface temperature trends: New evidence. *Geophys. Res. Lett.* **31**:L13207.
- Dudley, R. 1995. Extraordinary flight performance of orchid bees (Apidae: Euglossini) hovering in heliox (80% He/20% O₂). *J. Exp. Biol.* **198**:1065–1070.
- Dudley, R. 2000. *The biomechanics of insect flight: Form, function, and evolution*. Princeton University Press, Princeton.
- Dudley, R. and P. Chai. 1996. Animal flight mechanics in physically variable gas mixtures. *J. Exp. Biol.* **199**:1881–1885.
- Dupre, R. K. and S. C. Wood. 1988. Behavioral temperature regulation by aquatic ectotherms during hypoxia. *Can. J. Zool.* **66**:2649–2652.
- Fleishman, E., G.T. Austin, and A.D. Weiss. 1998. An empirical test of Rapoport's rule: Elevational gradients in montane butterfly communities. *Ecology* **79**:2482–2493.
- Frazier, M. R., H. A. Woods, and J. F. Harrison. 2001. Interactive effects of rearing temperature and oxygen on the development of *Drosophila melanogaster*. *Physiol. Biochem. Zool.* **74**:641–650.
- Frederich, M. and H. O. Portner. 2000. Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in spider crab, *Maja squinado*. *Am. J. Physiol.-Reg. I.* **279**:R1531–R1538.
- Galun, R. and G. Fraenkel. 1961. The effect of low atmospheric pressure on adult *Aedes aegyptii* and on housefly pupae. *J. Insect Physiol.* **7**:161–176.
- Garcia-Ramos, G. and M. Kirkpatrick. 1997. Genetic models of adaptation and gene flow in peripheral populations. *Evolution* **51**:21–28.
- Ghabrial, A., S. Luschnig, M. M. Metzstein, and M. A. Krasnow. 2003. Branching morphogenesis of the *Drosophila* tracheal system. *Ann. Rev. Cell Dev. Biol.* **19**:623–647.
- Gillooly, J. F., J. H. Brown, G. B. West, V. M. Savage, and E. L. Charnov. 2001. Effects of size and temperature on metabolic rate. *Science* **293**:2248–2251.
- Greenberg, S. and A. Ar. 1996. Effects of chronic hypoxia, normoxia and hyperoxia on larval development in the beetle *Tenebrio molitor*. *J. Insect Physiol.* **42**:991–996.

- Greenlee, K. J. and J. F. Harrison. 2004. Development of respiratory function in the American locust *Schistocerca americana* I. Across-instar effects. *J. Exp. Biol.* **207**: 497–508.
- Heinrich, B. 1975. Energetics of pollination. *Ann. Rev. Ecol. Syst.* **6**:139–170.
- Henry, J. R. and J. F. Harrison. 2004. Plastic and evolved responses of larval tracheae and mass to varying atmospheric oxygen content in *Drosophila melanogaster*. *J. Exp. Biol.* **207**:3559–3567.
- Hepburn, H. R., C. Youthed, and P. Illgner. 1998. Production of aerodynamic power in mountain honeybees (*Apis mellifera*). *Naturwissenschaften* **85**:389–390.
- Hicks, J. and S. Wood. 1985. Temperature regulation in lizards—effects of hypoxia. *Am. J. Physiol.* **248**:595–600.
- Hoback, W. W. and D. W. Stanley. 2001. Insects in hypoxia. *J. Insect Physiol.* **47**:533–542.
- Hopkins, A. D. 1938. *Bioclimatics: A science of life and climate relations*. United States Government Printing Office, Washington, D.C.
- Hosler, J. S., J. E. Burns, and H. E. Esch. 2000. Flight muscle resting potential and species-specific differences in chill-coma. *J. Insect Physiol.* **46**:621–627.
- Huey, R. B. 1991. Physiological consequences of habitat selection. *Am. Nat.* **137**:S91–S115.
- Huey, R. B., X. Eguskita, and M. E. Dillon. 2001. Mountaineering in thin air: Patterns of death and of weather at high altitude. In R. C. Roach, P. D. Wagner, and P. H. Hackett (eds), *Hypoxia: From genes to the bedside*, pp. 225–236. Kluwer Academic/Plenum Publishers, New York.
- Janzen, D. H. 1973. Sweep samples of tropical foliage insects—effects of seasons, vegetation types, elevation, time of day, and insularity. *Ecology* **54**:687–708.
- Janzen, D. H., M. Ataroff, M. Farinas, S. Reyes, N. Rincon, A. Soler, P. Soriano, and M. Vera. 1976. Changes in the arthropod community along an elevational transect in the Venezuelan Andes. *Biotropica* **8**:193–203.
- Jarecki, J., E. Johnson, and M. A. Krasnow. 1999. Oxygen regulation of airway branching in *Drosophila* is mediated by branchless FGF. *Cell* **99**:211–220.
- Jones, J. S., J. A. Coyne, and L. Partridge. 1987. Estimation of the thermal niche of *Drosophila melanogaster* using a temperature-sensitive mutation. *Am. Nat.* **130**:83–90.
- Josephson, R. K. 1981. Temperature and the mechanical performance of insect muscle. In Heinrich Bernd (ed.), *Insect thermoregulation*, pp. 20–44. Wiley & Sons, New York.
- Kalnay, E. and M. Cai. 2003. Impact of urbanization and land-use change on climate. *Nature* **423**:528–531.
- Karan, D., S. Dubey, B. Moreteau, R. Parkash, and J. David. 2000. Geographical clines for quantitative traits in natural populations of a tropical drosophilid: *Zaprionus indianus*. *Genetica* **108**:91–100.
- Keister, M. and J. Buck. 1961. Respiration of *Phormia regina* in relation to temperature and oxygen. *J. Insect Physiol.* **7**:51–72.
- Kingsolver, J. G. 1983. Thermoregulation and flight in *Colias* butterflies: Elevational patterns and mechanistic limitations. *Ecology* **64**:534–545.
- Kirkpatrick, M. and N. H. Barton. 1997. Evolution of a species' range. *Am. Nat.* **150**:1–23.
- Koning, J. and I. Jamieson. 2001. Variation in size of male weaponry in a harem-defence polygynous insect, the mountain stone weta *Hemideina maori* (Orthoptera: Anostostomatidae). *New Zeal. J. Zool.* **28**:109–117.
- Körner, C. 1999. *Alpine plant life: Functional plant ecology of high mountain ecosystems*. Springer, Berlin.
- Krasnov, B., D. Ward, and G. Shenbrot. 1996. Body size and leg length variation in several species of darkling beetles (Coleoptera: Tenebrionidae) along a rainfall and altitudinal gradient in the Negev Desert (Israel). *J. Arid Environ.* **34**:477–489.
- Lawton, J. H., M. Macgarvin, and P. A. Heads. 1987. Effects of altitude on the abundance and species richness of insect herbivores on bracken. *J. Anim. Ecol.* **56**:147–160.
- Lees, D. C., C. Kremen, and L. Andriamampianina. 1999. A null model for species richness gradients: Bounded range overlap of butterflies and other rainforest endemics in Madagascar. *Biol. J. Linn. Soc.* **67**:529–584.
- Lehmann, F. O. 2001. Matching spiracle opening to metabolic need during flight in *Drosophila*. *Science* **294**:1926–1929.
- Locke, M. 1958. The co-ordination of growth in the tracheal system of insects. *Q. J. Microsc. Sci.* **99**:373–391.
- Lomolino, M. V. 2001. Elevation gradients of species-density: Historical and prospective views. *Global Ecol. and Biogeogr.* **10**:3–13.
- Loudon, C. 1988. Development of *Tenebrio molitor* in low oxygen levels. *J. Insect Physiol.* **34**:97–103.
- Loudon, C. 1989. Tracheal hypertrophy in mealworms: Design and plasticity in oxygen supply systems. *J. Exp. Biol.* **147**:217–235.
- MacArthur, R. H. 1972. *Geographical ecology: Patterns in the distribution of species*. Harper & Row, New York.
- Mani, M. S. 1968. *Ecology and biogeography of high altitude insects*. Dr. W. Junk, The Hague.
- McCoy, E. D. 1990. The distribution of insects along elevational gradients. *Oikos* **58**:313–322.
- Monteith, J. L. and M. H. Unsworth. 1990. *Principles of environmental physics*. New York.
- Norry, F. M., O. A. Bublly, and V. Loeschcke. 2001. Developmental time, body size and wing loading in *Drosophila buzzatii* from lowland and highland populations in Argentina. *Hereditas* **135**:35–40.
- NRC Board on Atmospheric Sciences and Climate Committee. Panel, J. M. W., chair 2000. *Reconciling observations of global temperature change*, pp. 1–71. National Research Council, Washington, DC.
- Partridge, L., B. Barrie, K. Fowler, and V. French. 1994. Evolution and development of body size and cell size in *Drosophila melanogaster* in response to temperature. *Evolution* **48**:1269–1276.

- Porter, W. P. and D. M. Gates. 1969. Thermodynamic equilibria of animals with environment. *Ecol. Monogr.* **39**:227–244.
- Portner, H. O. 2001. Climate change and temperature-dependent biogeography: Oxygen limitation of thermal tolerance in animals. *Naturwissenschaften* **88**:137–146.
- Rahbek, C. 1995. The elevational gradient of species richness: A uniform pattern? *Ecography* **18**:200–205.
- Sanders, N. J. 2002. Elevational gradients in ant species richness: Area, geometry, and Rapoport's rule. *Ecography* **25**:25–32.
- Scaraffia, P. Y. and N. M. G. De Burgos. 2000. Effects of temperature and pH on hexokinase from the flight muscles of *Dipetalogaster maximus* (Hemiptera: Reduviidae). *J. Med. Entomol.* **37**:689–694.
- Schurmann, H. and J. F. Steffensen. 1992. Lethal oxygen levels at different temperatures and the preferred temperature during hypoxia of the Atlantic cod, *Gadus morhua* L. *J. Fish Biol.* **41**:927–934.
- Sibly, R. M. and D. Atkinson. 1994. How rearing temperature affects optimal adult body size in ectotherms. *Funct. Ecol.* **8**:486–493.
- Sømme, L. 1989. Adaptations of terrestrial arthropods to the alpine environment. *Biol. Rev.* **64**:367–407.
- Stalker, H. D. and H. L. Carson. 1948. An altitudinal transect of *Drosophila robusta* Sturtevant. *Evolution* **2**:295–305.
- Stevenson, R. D. 1985. Body size and limits to the daily range of body—temperature in terrestrial ectotherms. *Am. Nat.* **125**:102–117.
- Thiele, H. U. and H. Kirchner. 1958. Über die Körpergröße der Gebirgs- Flachlandpopulationen einiger Laufkäfer. *Bonn. Zool. Beitr.* **9**:294–302.
- Vogel, S. 1994. *Life in moving fluids: The physical biology of flow.* Princeton University Press, Princeton, N.J.
- U.S. Standard Atmosphere. 1976. U.S. Government Printing Office, Washington, D.C.
- Walsberg, G. E. 1992. Quantifying radiative heat gain in animals. *Am. Zool.* **32**:217–224.
- Ward, M. P., J. S. Milledge, and J. B. West. 2000. *High altitude medicine and physiology.* Arnold, London.
- West, J. B. 1996. Prediction of barometric pressures at high altitudes with the use of model atmospheres. *J. Appl. Phys.* **81**:1850–1854.
- Wigglesworth, V. B. 1954. Growth and regeneration in the tracheal system of an insect, *Rhodnius prolixus* (Hemiptera). *Q. J. Microsc. Sci.* **95**:115–137.
- Wigglesworth, V. B. 1972. *The principles of insect physiology.* Chapman and Hall, London.
- Withers, P. C. 1981. The effects of ambient air pressure on oxygen consumption of resting and hovering honeybees. *J. Comp. Physiol. B* **141**:433–437.
- Woods, H. A. 1999. Egg-mass size and cell size: Effects of temperature on oxygen distribution. *Am. Zool.* **39**:244–252.
- Woods, H. A. and R. I. Hill. 2004. Temperature-dependent oxygen limitation in insect eggs. *J. Exp. Biol.* **207**:2267–2276.