Insects in Fluctuating Thermal Environments

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Abstract

All climate change scenarios predict an increase in both global temperature means and the magnitude of seasonal and diel temperature variation. The nonlinear relationship between temperature and biological processes means that fluctuating temperatures lead to physiological, life history, and ecological consequences for ectothermic insects that diverge from those predicted from constant temperatures. Fluctuating temperatures that remain within permissive temperature ranges generally improve performance. By contrast, those which extend to stressful temperatures may have either positive impacts, allowing repair of damage accrued during exposure to thermal extremes, or negative impacts from cumulative damage during successive exposures. We discuss the mechanisms underlying these differing effects. Fluctuating temperatures could be used to enhance or weaken insects in applied rearing programs, and any prediction of insect performance in the field—including models of climate change or population performance—must account for the effect of fluctuating temperatures.

INTRODUCTION

Fluctuating temperatures (FTs): a generic term that refers to any discontinuous thermal regime that occurs short-term (intragenerational)

Thermal performance curves (TPCs): the (usually asymmetric) relationship between temperature and performance of an ectotherm

Insects drive terrestrial ecosystems, and—as they are small ectotherms—their biology is closely linked to environmental temperature. Temperature determines insect survival, population dynamics, and distribution (1, 23, 24), and thus their responses to climate change (4, 22, 38). Temperature in the field fluctuates, and the impacts of this variation have been recognized in areas as diverse as forensic entomology (18, 53), thermal tolerance physiology (9, 80, 96), biocontrol (13, 28), insect-mediated pollination (98, 123), disease vector biology (73, 87), and simulated climate warming studies (4, 10, 56, 116, 125).

Researchers in the early 1900s reported that insects grow faster under fluctuating temperatures (FTs) compared with constant temperatures (CTs) (34, 100), and early reviews (25, 93) acknowledged that FTs reflected natural conditions better than CTs. In the context of development, these early reviews already pointed out that the "nonlinear temperature-velocity relationship" (93) means that FT treatments should be "normal" whereas CT insect development studies were essentially conducted under "abnormal" conditions (25). In the 1970s, it became apparent that FTs improved thermal tolerance of insects over those exposed to CTs (17, 82) and that fitness could be greater in FTs (6). Research on FTs resurged in the early 2000s, particularly in the context of insect cold tolerance (75, 83, 96). Presently, FTs are under extensive investigation in the context of climate change and the extrapolation of laboratory studies to the field, with the goal of incorporating thermal variability and extreme events in ecological and physiological studies (4, 109, 116).

Here, we synthesize the disparate work on the impacts of FTs on insects, emphasizing the need for particular care when interpreting results derived from static designs. We give an overview of the methods and approaches that have been used to explore the differences between insect responses to FTs and CTs and focus on general principles and responses rather than specific organisms.

THE BIOLOGICAL IMPACTS OF TEMPERATURE

Thermal Variability in the Environment

The environmental temperature in terrestrial habitats fluctuates on multiple time scales (36, 80). The amplitude of daily thermal fluctuations varies by season and habitat (92) and can be more than 30°C (102). At high latitudes and altitudes, these fluctuations may cross a species' freezing threshold at any time of year (80, 112). Likewise, temperatures fluctuate above thresholds for heat shock year-round in hot climates (47). Weather patterns that occur over multiday periods can modulate the amplitude of diel temperature cycles within a season (80). The occurrence and amplitude of daily FTs can also be modulated by habitat (45) and microhabitat (118). Some examples are the insulating effect of snow cover or thermal inertia from soil, trees, or litter (36); however, these microclimate temperatures are generally not well captured by global-scale weather data sets. Thus, individual insects may experience FTs on a scale that fits within the developmental period and life span of even short-lived species. As a consequence, they must constantly adjust their physiology to changing thermal conditions.

Temperature Effects in Biology

In ectothermic animals like insects, thermal performance curves (TPCs) are nonlinear and asymmetric (1) (**Figure 1**). Temperature shifts will thus result in uneven effects depending on whether the temperature varies above or below the optimal temperature (99). Even at permissive

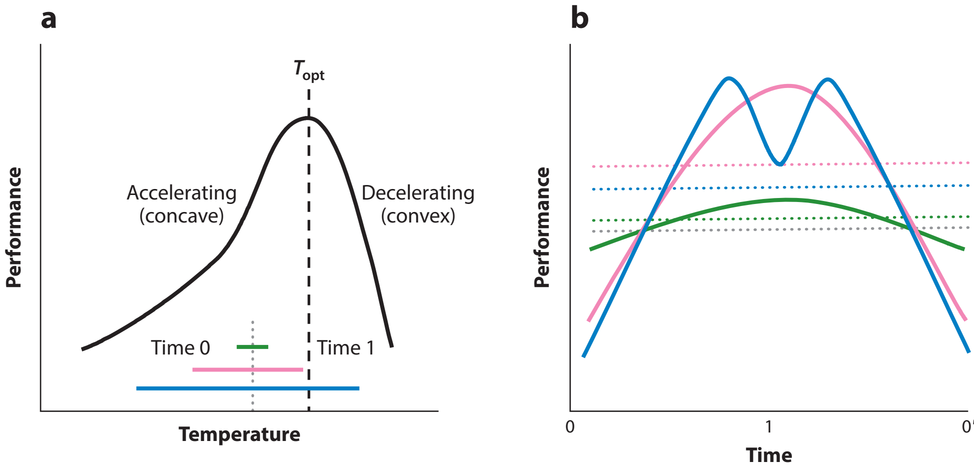
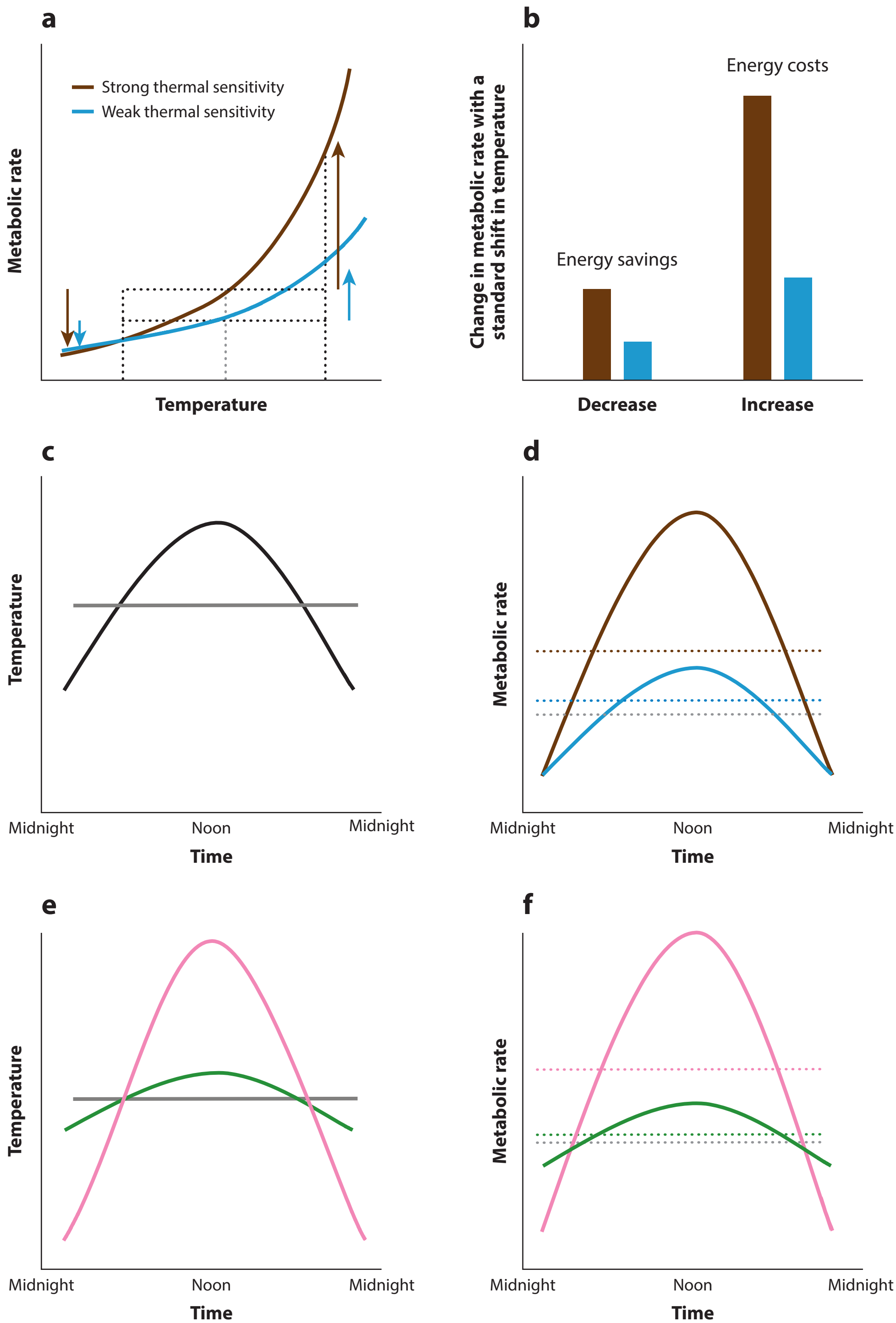


Figure 1

Relationship between performance and temperature of an insect. (*a*) Thermal performance curve, showing accelerating temperature-performance relationship below an optimal temperature (T_{opt}) and decelerating relationship above T_{opt}. Horizontal lines indicate the spans of three symmetrical fluctuating temperature regimes (between time 0 and time 1) with the same mean (indicated by *gray* dotted line). Note that the regime depicted in blue (*bottom*) spans temperatures above T_{opt}. (*b*) Change in performance trait shown in panel *a* over the course of a single cycle (from time 0, at the minimum of the cycle, to time 1, at the top of the cycle, and back to the minimum of the cycle at time 0′) of the three temperature regimes shown in panel *a*, with the means displayed as dotted lines (*gray* = constant temperature). Note that average performance (dotted lines with the corresponding colors) declines if the temperature spans temperatures above T_{opt} (*blue*).

temperatures, an animal can pass physiological thresholds during a thermal cycle, reaching critical temperatures such as the critical thermal minimum (CT_{min}) or maximum (CT_{max}). At extreme temperatures, the temperature-process relationship can change abruptly; for example, proteins are denatured by heat, and water freezes at low temperatures (23, 74). The asymmetry of TPCs places the maximum rates of TPCs close to the upper thermal limits (1, 81, 99); thus, small increases in temperature may push insects over the CT_{max} (**Figure 1**). At low temperatures, the changes in rates are slower, and therefore there is less chance of hitting abrupt limits. In the concave (accelerating) part of the TPC, the total output of a rate process in FT-exposed insects will exceed that predicted for CT-exposed insects with an equivalent mean (45, 57, 81). This disproportionate effect is exacerbated by FTs with greater amplitude (**Figure 1**). The opposite will be observed in the convex (decelerating) part of the TPC. This phenomenon, known as Jensen's inequality (57), explains many of the discrepancies between FT and CT experiments. The physiological response to FTs, such as metabolic rate changes, are asymmetrical (118), with limited effects of decreasing temperatures and greater effects of increasing temperatures (57, 81) (**Figure 2**). The discrepancies between FT and CT experiments will depend on the degree of thermal sensitivity of the process, with lesser effects of FTs when thermal sensitivity is weaker (i.e., smaller degree of curvature), and the amplitude of the thermal cycle: Larger amplitudes will have a greater impact (45, 99) (**Figure 2**). Although this means that development should be faster under FTs than CTs, the energetic costs incurred by a fasting ectotherm in the warming part of a daily cycle will be greater than the energetic savings resulting from the cooling part, especially in thermally sensitive species (118) (**Figure 2**); thus, fluctuating environments are more energy demanding than static environments.

Critical temperatures CT_{min} and CT_{max}: low and high temperatures at which motor function stops and coordination is lost



a
Strong thermal sensitivity
Weak thermal sensitivity
Metabolic rate
Temperature
b
Energy costs
Energy savings
Change in metabolic rate with a standard shift in temperature
Decrease
Increase
c
Temperature
Midnight
Noon
Midnight
Time
d
Metabolic rate
Midnight
Noon
Midnight
Time
e
Temperature
Midnight
Noon
Midnight
Time
f
Metabolic rate
Midnight
Noon
Midnight
Time

DESIGN AND INTERPRETATION OF EXPERIMENTS INCORPORATING FTs

The term fluctuating temperatures covers a range of time scales and temperature transitions. Insects can respond to these fluctuations in ways stretching from hardening responses (on a scale of minutes) to evolutionary responses over geological time. Here, we focus on FTs that recur more than once within a single developmental stage, although FT experiments may apply those fluctuations throughout development. The FT literature contains almost as many exposure regimes as it does experiments, from the simple use of two alternating temperatures to use of more sophisticated simulations of the daily temperature patterns. A glimpse of the diversity of these approaches is summarized in **Figure 3** and in **Supplemental Table 1** (follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**).

Supplemental Material

The temperatures included in an FT experiment will be dictated by the purpose of the study and by the tolerance of the insect. An initial decision is whether the fluctuations should be within the permissive range—appropriate if the goal is to understand diel thermal cycles (62, 87)—or include extreme temperatures—appropriate if the goal is to understand the consequences of crossing physiological thresholds (80, 83). Although it may be sufficient to have simple step-function transfers from one temperature to another, ramped temperature changes, or even curvilinear temperature regimes, will better reflect the natural environment (**Figure 3**). These temperature regimes will differ in the amount of time spent outside the permissive temperature range.

Many FT experiments use a CT equivalent to the mean of the FT as a control. However, controls must account for the amount of time spent at high or low temperatures and the nonlinear effects of FTs on physiological rates. Marshall & Sinclair (80) suggested a matched cold design (also adaptable to heat experiments), which includes a control for the effect of a single exposure equivalent to one cycle of the regime, and a control that exposes the insect to the low temperature for an amount of time that is equivalent to the total cumulative amount of time of exposure to cold. This design limits the choice of temperatures to those that the insect can survive for a long period. Because FT experiments are often conducted over multiple cycles, experimental animals are ageing: An insect that is exposed to ten daily cycles is not only responding to the repeated cycles but is also ten days older than an animal exposed on the first day. Simple preliminary experiments should be carried out to rule out any putative ageing effect. Finally, variables other than temperature fluctuate in the wild, and these may provide important cues for physiological responses. For example, photoperiod and humidity cycles may be as important as temperature

Figure 2

The effect of Jensen's inequality, thermal sensitivity, and cycle amplitude on the relationship between temperature and metabolic rate under fluctuating temperatures. (*a*) Representative curvilinear relationships between metabolic rate and temperature for species with strong (*brown*) and weak (*blue*) thermal sensitivity. Dotted lines indicate a standard shift in temperature above and below a mean (*gray*), and arrows indicate the magnitude of the shift in metabolic rate. (*b*) Energy decreases (savings) or increases (costs) in response to a standard shift in temperature up or down from a mean for the curves in panel *a*. (*c*) Hypothetical daily temperature cycle (*black*) or constant temperature (*gray*). (*d*) Instantaneous metabolic rate of thermally sensitive (*brown*) and thermally insensitive (*blue*) phenotypes from panel *a* under the temperature regimes shown in panel *c*. Dotted lines indicate mean rate across the day, compared with the constant temperature (*gray*). (*e*) Hypothetical thermal cycles of large (*pink*) and small (*green*) amplitude, or constant temperature (*gray*). (*f*) Instantaneous metabolic rates of a single phenotype under the temperature regimes shown in panel *e* compared with constant temperature (*gray*). Note that although metabolic rate is used for this example, any curvilinear process will follow a similar pattern if temperature fluctuations are within the accelerating portion of the curve shown in **Figure 1** (see 99, 118).

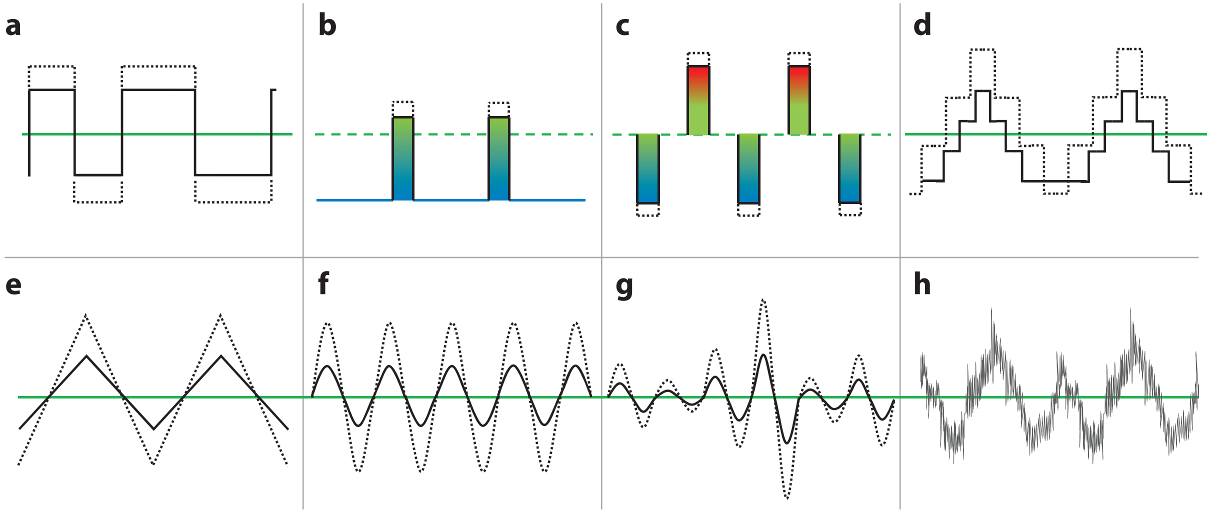


Figure 3

The diversity of fluctuating temperature (FT) treatments. (*a*) Two temperatures alternating around a constant mean (continuous *green* line). These protocols use rapid step transitions. (*b*) Interruption of a prolonged cold stress (*blue*) by repeated bouts at optimal temperature (*green*). (*c*) Repeated exposures to damaging temperatures simulating the effect of heat (*red*) and cold (*blue*) waves. In panels *b* and *c*, the dashed green line represents the optimal temperature. (*d*) Multiple step transitions regime around a mean (*green* line) used to simulate complex diel cycles or nature-mimicking thermoperiods. (*e*) FTs with controlled gradual transitions (ramp) around a mean (*green* line). (*f*) Sine-like wave thermal cycles (night-days) can be symmetric or asymmetric around a mean (*green* line). (*g*) Stochastic sinusoidal or diel thermal variations. (*h*) Field temperature variations. For all these treatments (panels *a*–*g*), different amplitudes (dotted *black* lines), durations, and frequencies of temperature breaks can be applied.

(8, 64). Fortunately, these cues are often synchronized with temperature cycles, so laboratory procedures can fairly easily reproduce this synchronicity (e.g., 126).

EFFECTS OF FTs ON LIFE HISTORY TRAITS AND FITNESS

Development

Fluctuating temperatures that extend to deleterious high or low temperatures can allow development outside the temperatures where it would normally occur (37, 46, 76, 90). However, FTs using deleterious temperatures generally delay development compared with development at optimal CTs (46, 63). These delays are likely a consequence of direct cold or heat injuries and of the costs of subsequent physiological and biochemical repair (24, 43). By contrast, FTs that remain within the permissive thermal range can result in diverse responses, including accelerated development (2, 13, 44, 65, 66), slower development (25, 42, 66), or no change in developmental rate (65). One explanation of this variation in responses is that the effect of FTs on the development may depend on the thermal mean that is used and its proximity to developmental thresholds (65). Accelerated development appears to be the norm if the lower temperature of the FT is not injurious but falls below a species' thermal threshold for development (93). Finally, the effect of FTs on development time also depends on the amplitude of the variation (14, 42, 46, 66), likely because of Jensen's inequality. For example, *Aedes aegypti* mosquitoes reached pupation four days faster when reared under large (18.6°C), rather than small (7.6°C), daily FTs (14).

Morphology

The body size, shape, and symmetry of imagoes integrate the stresses experienced during development and can thus provide a measure of developmental stability. Perhaps the most subtle morphological impact of developmental stress is fluctuating asymmetry (FA) (7). Early studies comparing FA of CT- versus FT-reared *Drosophila melanogaster* were contradictory: FTs led to both reduced (5) and increased (11) asymmetry. Temperature cycles that included a cold stress during development reduced FA in the noctuid moth *Helicoverpa punctigera*, although the experimental design did not allow for the effects of FTs and low temperatures per se to be teased apart (54). FTs that approach thermal limits can also result in increased variability of morphological traits (89). Thus, FTs that encompass deleterious temperatures appear to increase phenotypic variation and developmental instability.

Fluctuating asymmetry (FA): a pattern of deviation from bilateral morphological symmetry

The temperature-size rule predicts that development at higher temperatures should result in small insects (3). If, like other rate processes, the temperature–final size relationship is curvilinear, Jensen's inequality would predict a disproportionate influence of high temperatures under FTs (81, 99). Indeed, FTs with large thermal amplitudes reduced the pupal size of *Manduca sexta* (65) and thorax size, wing size, and body weight (35, 42, 89, 90) of drosophilids. Reduced size is likely mediated by an energy use–structural allocation trade-off (more energy is diverted to metabolism and maintenance at higher temperatures) and earlier maturity, possibly because elevated temperatures affect the differentiation rate of the cells more than their growth rate (114). However, the information on the effects of FTs on cell differentiation is scarce, but see Reference 71.

Life Span

Fluctuating temperatures have been reported to increase (33, 42), decrease (13, 16), or have no effect (66) on life span. These discrepancies likely arise from the diversity of species and approaches, and a systematic comparative approach (e.g., 84) could yield a more meaningful signal. If injury is incurred during the high or low temperature portions of FTs, a straightforward trade-off between damage repair and somatic maintenance could reduce longevity. However, *Alphitobius diaperinus* exposed to 5°C alternating with 20°C showed a large overshoot in oxygen consumption associated with increased reactive oxygen species (ROS) production during the warm period (72), which is consistent with the theoretical role of ROS production as an underlying mechanism of ageing (104). From this, we predict that FTs that do not lead to life span reduction would not increase ROS production. Because metabolic rate (and presumably ageing) fluctuates in a curvilinear fashion throughout the FTs (10), FTs may decouple physiological age from chronological age, yielding a complexity of results consistent with the observed discrepancies in FT effects on longevity.

Fecundity

Reproductive output is a central component of fitness and can thus be used as part of a measure of the fitness consequences of FTs (79, 95). FTs increased reproductive output in some studies (95), but this effect appears to be dose dependent. For example, FTs within the optimal thermal zone lead to a positive relationship between amplitude of FTs and egg production in *Ceratitis capitata* (107), whereas FTs that encompass stressful temperatures reduce fecundity (16, 79). Increasing number of cold exposures (0°C) decreased reproductive output of female *D. melanogaster* (79), as did a single, one- to three-day exposure to a suboptimal temperature (39). Similarly, fewer eggs were produced by *Zeiraphera canadensis* moths in a 10–25°C FT regime compared with controls at 20°C (25°C being supraoptimal for this species) (16).

Fluctuating acclimation regime (FAR): a thermal acclimation treatment that uses fluctuating preexposure temperatures for conditioning individuals

Constant acclimation regime (CAR): a thermal acclimation treatment that uses constant preexposure temperatures for conditioning individuals

Stressful temperatures impair oocyte development (48) and decrease mating success (27), sperm production, and sperm viability (97), so the mechanisms underlying a decrease in reproductive output after stressful temperatures are easy to envisage. However, it is unclear whether these different processes have differing thresholds or responses to FTs, and this should be a topic for future research. The mechanisms underlying increased reproductive investment under FTs (107) may be as simple as an effect of Jensen's inequality on reproductive physiology or may involve more complex signaling pathways; these have not been investigated. Similarly, the duration of the FT effect has not been well characterized: It is as yet unclear whether FTs lead to a lifetime change in reproductive investment or a transient change that can be modified with repair and recovery.

EFFECTS OF FTs ON THERMAL TOLERANCE

Effects of FTs During Acclimation

Most laboratory acclimation experiments on insects use CTs, even though the studied organism would typically experience thermally variable environments. Egg-to-adult development under fluctuating acclimation regimes (FARs) increased *D. melanogaster* cold tolerance (85), and the heat tolerance of drosophilids (9, 101) and lycaenid butterflies (44), compared with development under constant acclimation regimes (CARs). Acclimation of adult stages under FTs improves thermal tolerance of *D. melanogaster* (62) and the tephritid *Dacus tryoni* (82). The response to FARs is dependent on the mean (44) and on the amplitude (107) in a species- and stress-specific manner: Small FT amplitudes increased cold tolerance of *Ceratitis capitata*, but heat tolerance was greatest under high-amplitude FTs (107). Antarctic springtails (*Cryptopygus antarcticus*) had greater cold tolerance and plasticity in thermally variable compared with buffered microcosms, which suggests that FTs may drive thermal tolerance in the field (50). By contrast, acclimation of fall field crickets, *Gryllus pennsylvanicus*, was unaffected by the amplitude or predictability of FARs (84), suggesting that FARs are not uniformly effective at increasing thermal tolerance.

FTs Can Mitigate Prolonged Low-Temperature Stress

Chilling at temperatures not associated with ice formation is lethal to many insects (23, 74), and these injuries can be reduced or avoided if the cold period is interrupted with brief exposures to warmer temperatures. For example, Chen & Denlinger (19) reported that pharate adults of the flesh fly *Sarcophaga crassipalpis* could not tolerate a 2-h exposure to −10°C after being held at 0°C for 20 days. But, when the 20 days of exposure to 0°C was interrupted by a single 6-h pulse at 15°C on day 10, 53% of insects survived a 10-h exposure to −10°C. This was among the first reports of a recharge process under FTs (19). The beneficial effect of interrupting prolonged cold exposure with warm periods (also referred to as fluctuating thermal regime, FTR) has since been reported for Hemiptera (67), Orthoptera (58), Diptera (19, 75, 79), Coleoptera (96), Hymenoptera (28, 32, 123), Lepidoptera (8, 64, 111), and Collembola (83), suggesting that the response is highly conserved across taxa. Warm interruptions as short as 5 min can improve cold survival (123), and increased duration of the warm phase usually results in improved survival (58, 83, 123), to a point where any effect of chilling becomes negligible (30). The effect of warm interruption is temperature dependent, although the warmest temperatures do not necessarily yield the best survival gains (83). Increased frequency of warming pulses also promotes longer survival (32, 58, 83, 123).

Reduced cold mortality under FTs is probably not due to a reduction of cumulative chill injury, as the effects persist even when strictly equivalent cold doses are compared (8, 30, 70, 96). Alternatively, it seems likely that chilling injury is repaired during the warming intervals (32, 67, 96) (see Mechanisms Underlying the Response to FTs). Interestingly, the benefits of FTs appear to apply only to freeze-avoiding and chill-susceptible species: Repeated freeze-thaw is damaging to freeze-tolerant species (80). In addition, long warming interruptions can lead to deacclimation and loss of cold tolerance (103, 111). For example, overwintering emerald ash borer (*Agrilus planipennis*) prepupae irreversibly lost their cold tolerance after exposure to +10°C for more than a week, reducing survival of subsequent cold exposures (103).

Heat shock response: the physiological and molecular responses to a brief exposure to high temperatures; usually includes the synthesis of heat shock proteins

FTs During Heat Stress

Insects generally have a well-developed heat shock response (43), and this is clearly relevant to FTs that extend above the optimum range. Although upper thermal limits are in dangerous proximity to optima because of Jensen's inequality (81), there is capacity for this threshold to shift, with return to permissive temperatures, as well as for repair to occur. As with low temperatures, FTs allow development and survival under conditions that include high temperatures that might otherwise be lethal (37, 46, 76, 108). For example, *D. melanogaster* cannot develop at 33°C but can develop and survive if the temperature fluctuates from 33°C to 13°C (42). Thus, FTs can increase thermal range when recovery is possible.

Prior exposure to high temperatures improves survival of insects on hot days in the field (21, 86), which implies that insects may be able to survive in the field at higher temperatures than predicted from laboratory experiments conducted under CTs. Because of the asymmetrical shape of the TPC (1) (**Figure 1**), there is more chance of hitting abrupt limits and irreversible thresholds at high temperatures. Thus, heat damages may not be as easily repaired as cold damages. Until recently, much less was known about the impacts of repeated heat stress and FTs that span high temperatures compared with repeated cold stress and cold FTs. Recent data support the notion that extreme heat events, even of short duration or when occurring only once, are highly detrimental for species' performance and survival and that averaging daily temperature will not capture these effects (4, 56, 88, 116, 125).

MECHANISMS UNDERLYING THE RESPONSE TO FTs

Physiological Correlates of Fluctuating Acclimation Regimes

FARs generally promote cold tolerance compared with cold CARs (see Effects of FTs on Thermal Tolerance), possibly because the warm intervals allow physiological changes that are not otherwise possible. Membrane lipid composition shifts after the first temperature cycle between 5°C and 20°C in *Orchesella cincta* springtails (115), but these changes were not consistent with homeoviscous adaptation (51). In most cases, chaperone proteins appear to be upregulated more under FARs than under CARs, potentially allowing increased protection of proteins against thermal shock (2, 117). However, lycaenid butterflies exposed to multistep FT regimes (daily means of 17.7°C or 23.7°C) showed the opposite response: a decrease in HSP70 expression in insects exposed to FTs (44). These discrepancies may reflect variation in the degree to which the thermal conditions are physiologically stressful and may arise from a focus on basal heat shock protein (HSP) expression, which may be a poor reflection of the real (stress-induced) capacity for protection from thermal stress (43).

Cold tolerance of insects is usually associated with the accumulation of polyols and sugars (74), so it may be expected that the improvement in cold tolerance under FARs would be accompanied by increased concentrations of these cryoprotectants. This has been observed in Hemiptera (68) and Orthoptera (117). *Dendroides canadensis* beetles accumulate antifreeze proteins under short-day thermoperiods in the absence of any light-dark cycle (55). This may reflect a role of FARs in stimulating acclimation responses for organisms that do not receive reliable photoperiodic cues because they are under bark (as in this case) or in the soil. Finally, increased thermal variance during FAR treatment reduced maximum metabolic rate in *Tenebrio molitor* (10), suggesting that FARs may drive metabolic depression, as reported for overwintering lepidopteran larvae (118). Even if various physiological correlates of FARs have been described, the molecular mechanisms underlying the responses to FARs have not yet been investigated.

Physiological Responses to FTs: Repair and Protection During the Warm Phase?

FTs could improve cold survival by allowing physiological preparation for subsequent cold exposures in a manner similar to the rapid cold hardening response (105). However, increased survival of prolonged cold is most likely improved by FTs because damage accrued during the cold phase of the temperature cycles is then repaired during warming episodes (31, 32, 67, 83, 96). Chilling in insects is accompanied by a loss of ion homeostasis (69, 78). When rewarmed, insects must therefore reestablish ion balance—which is energetically expensive (78)—and repair damage caused by osmotic and ionic stress. Under FTs, chill-susceptible *Pyrrhocoris apterus* and *A. diaperinus* reestablished ion balance during each warm spell, which likely increased the duration of survival over counterparts exposed to constant cold (67).

Protein unfolding or misfolding is another stress associated with thermal extremes and one that is managed in cells by the upregulation of HSPs, particularly those in the 70-kDa family (43). The *hsp70* gene and/or its protein concentration was upregulated under FTs compared with CTs in Coleoptera (121), Hymenoptera (31), and Lepidoptera (8). However, although *hsp70* mRNA abundance increased 1,000-fold during warming phases in *P. apterus*, no significant change was found at the protein level (110), presumably because the 2-h warming intervals were too short for translation to occur. Other HSP families are upregulated in response to FTs (124) and might thus also contribute to FT response. Whether HSP expression during the FTs is a reaction to stress or an adaptive protective effect is not yet clear, but experiments using RNA interference or transgenic overexpression could help determine the role of HSPs in phenotypes associated with FTs. HSPs would also be expected to play an important role in repair of (and protection against) damage in FTs that span high temperatures, but to our knowledge, this has not yet been examined.

Compatible solutes, such as sugars, polyols, or free amino acids, have a range of protective properties, such as detoxification or stabilization of proteins and membranes (120). These molecules might therefore play a role in repair of damage (or protection from future damage) during FTs. Increased glucose concentration is a common feature of insects exposed to repeated cold (106), and polyols (especially glycerol) accumulate in response to FTs in Diptera and Coleoptera (70, 91). Although the cryoprotectant role of polyols and sugars is well established (23, 74), the role of amino acids is less well understood. Most free amino acids are accumulated by *Aphidius colemani* in response to cold CTs (29), whereas the free amino acid pool decreased during the warm periods of FTs in *A. diaperinus* (70) and *A. colemani* (29), suggesting that warming intervals reactivate the utilization of amino acids for protein synthesis and energetic purpose. Energy metabolism is an alternative role for many putatively cryoprotective solutes. Although ATP supply does not always decline in the cold (26, 77), any depletion can certainly be regenerated during the warm spells in FTs (40) (but see 26), and sufficient ATP supply may be required for energy-demanding repair and recovery processes. Several studies have reported increased metabolic rates during the warm period of FTs (8, 72, 122), and proteins related to energy metabolism are upregulated during the warming periods in *A. colemani* (31). Such increased metabolism might be associated with generation of free radicals (ROS) and oxidative stress. However, cold-induced oxidative stress (measured as the ratio of reduced to oxidized glutathione) actually decreased during the warm phase of FTs in *A. diaperinus* (72), likely because of increased function of antioxidant enzymes at warmer temperatures. Together, these data suggest an active regulation of ion homeostasis, chaperone machinery, energy metabolism, and respiration during FTs, particularly during warm periods.

APPLICATIONS AND IMPLICATIONS OF FTs

Exploiting the Protective Effects of FTs

Because FTs that rewarm insects for brief periods during cold exposure mitigate many of the negative impacts of low temperatures, they have obvious applications in the context of cold storage of beneficial insects (28). For example, storage at constant 2°C for 20 days reduced survival to less than 40% in several aphidine parasitoids, whereas storage at FTs (2°C interrupted by spells at 20°C) allowed maintenance of a high survival (equal to untreated control), thus improving stockpiling and mass-rearing efficiency (28). Storage under FTs also holds applications for mass rearing of insects for pollination (98, 123) and potential applications for sterile insect technique (21). FTs could be exploited to deacclimate insects (103) and thus to reduce cold tolerance of pests as part of a thermal quarantine procedure. Similarly, the negative impacts of repeated cold or heat stress (80) could be leveraged to control stored product pests (59), but this has not been well explored.

Implications of FTs for Prediction and Management

It is clear that the survival, development, and performance of insects under FTs are poorly predicted by CTs. Nevertheless, many approaches for predicting the performance and survival of insects are dependent on data gathered using CTs or assume simple linear relationships between biological rate processes and temperature. For example, degree-day models underpin estimates of development time used to forecast population dynamics in agriculture (119). However, such models could be flawed, as they do not account for Jensen's inequality and therefore underestimate the contribution of temperatures above the mean to development and fecundity. This implication of FTs for degree-day models is well recognized in forensic entomology (18, 53). Just as FTs affect development, they also affect overwinter survival and thus predictability of agricultural pest outbreaks or species distribution modeling (113). In addition, because FTs lead to changes in thermal tolerance over time (62, 117), predictions of winter mortality derived from static estimates from CTs may be flawed. Régnière & Bentz (94) developed a model for mountain pine beetle (*Dendroctonus ponderosae*) mortality that recognized the dynamic nature of thermal tolerance, and such an approach could likely be utilized to reflect the cumulative impact of FTs on survival and performance. FTs modify life history traits, population dynamics, and immunocompetence of disease-vector insects (73, 87), and large FTs (18°C swings around 20°C) accelerate virus transmission by mosquitoes (15). Thus, it is clear that the performance effects of FTs on insects can have a broad societal significance.

FTs AND INSECT RESPONSES TO CLIMATE CHANGE

Ongoing global climate change is predicted to lead to increases in mean temperature, increased variance around that mean, and an increased incidence of transient extreme temperatures (41, 49, 116). The distribution and phenology of insects have been responding apace with this changing climate (20, 22). However, the role of thermal variability has not been regularly included in experimental studies and predictive models (52, 109), despite the relevance of variability to model outputs (60, 88, 118). The asymmetric nature of TPCs means that increased temperature places ectotherms closer to their upper thermal limits (38, 61), and an increase in variability exacerbates this risk (88) such that the impacts of climate change are best described by the interaction between mean temperature and variability (109, 116). For insects, this mean × variance interaction is even more complex. FTs alter fitness components, including fecundity, longevity, and body size, while affecting thermal plasticity, stress survival, recovery, and response to transient extremes. Importantly, these traits vary with FTs in a curvilinear, or even threshold, fashion that is not accounted for by bioclimatic envelope models and is captured poorly in the current generation of mechanistic models (12, 116). Thus, models of insect responses to climate change that are parameterized from data sets gathered under CTs (or that assume no change in the variance of thermal regimes) may contain systematic errors when compared with the real world (52). Although there is much to learn about how insects respond to FTs, we suggest that initial models that incorporate thermal variance (109, 116) could provide some guidance for the exploration of insect responses to FTs in a context directly usable to drive policy and management.

CONCLUSIONS

Insect responses to FTs contrast with responses to CTs at multiple levels of organization, from physiology and stress tolerance to life history traits and fitness. This divergence has important implications not only for the design and interpretation of thermal biology studies but also for predicting responses to climate change. We conclude that CTs are an unrealistic approach for studying the thermal responses of insects that typically occur in thermally variable environments. As a result, FTs should be incorporated into predictive models of growth, performance, survival, and climate change responses and play a central role in the design of all laboratory studies in insect thermal biology.

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