Insects at Not So Low Temperature:
Climate Change in the Temperate Zone and Its Biotic Consequences

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X.1. Introduction

Herein, we consider insects in the temperate zone where, in central and eastern continental land masses, favorable summers are interspersed with inamicable, often lethal, winters. At latitudes higher than 30°, fitness consists of the ability to exploit the warm season through growth, development and reproduction, the ability to avoid or mitigate the effects of winter cold through dormancy or migration, and the ability to make a timely transition between summer and winter lifestyles. Fitness is then defined by performance integrated through all four seasons, not just by a measure of performance in a single environment characteristic of a single time of year.

Below we discuss the geographical and seasonal patterns in light and temperature in the temperate zone, how climate change is and will affect these patterns and the actual and potential biotic responses by insects to climate warming. Physiological processes lie at the level of integration between the environment and the gene and are important in regulating the acquisition, assimilation and allocation of resources, in regulating growth, development and reproduction and in maintaining homeostasis in variable environments. Environmental change elicits a physiological response, either through non-genetic change within individual animals (homeostasis and phenotypic plasticity) or through genetic change in animal populations (evolution). Mechanism matters. Physiological processes enable animals to maximize survivorship and reproduction and are primary determinants of fitness in environments that vary in time and space. In temperate regions, the major biotic patterns of response to climate change are clear: Animals are expanding their ranges poleward and are altering the timing of their seasonal development. Herein, we consider established effects of day length and temperature on development, reproduction and dormancy and how these effects impede or facilitate response to rapid climate change at both the phenotypic and genetic (evolutionary) level.

X.2 Heat and Light on a Rotating Earth

X.2.1 How Does Seasonality Change with Geography?

Because of the 23° tilt of the earth's axis of daily rotation relative to the plane of its annual rotation about the sun, northern and southern hemispheres experience opposite periods of summer and winter. Also because of this tilt, not only is winter day length shorter at higher than at lower latitudes (Fig. 1A), but also the angle of incident winter sunlight is more acute and imparts less heat per hour of daylight. Consequently, the latitudinal gradient in climate is

primarily one of winter cold rather than summer heat (Fig. 1B). Concomitantly, cold tolerance or lower lethal limits in insects are more closely associated with latitude than are heat tolerance or upper lethal limits (Addo-Bediako *et al*., 2000; Zani *et al*., 2005). As one proceeds polewards, spring arrives later and fall arrives earlier. Hence, the length of the favorable season declines regularly with increasing latitude. For example, in Figure 1B, the 15°C isotherm declines from 10½ months at 30°N to 3 months at 48°N. When air masses encounter mountain ranges, they rise, expand and cool so that the length of the favorable season also declines with increasing altitude (MacArthur, 1972; Critchfield, 1974). In eastern North America, the number of freeze-free days decreases by about 9.3 days with every degree of increasing latitude and 94 days with every 1,000 m of increasing elevation (Bradshaw 1976). Seasonal activities of temperate insects are therefore intimately related to the coming of spring and fall and to the length of the growing season. As the length of the growing season and the coming of spring and fall vary with geography, so also do the optimal times to initiate growth, development, reproduction, dormancy or migration.

X.2.2. How do Insects Time Their Seasonal Activities?

A wide variety of animals use the length of day as an anticipatory cue (photoperiodism) to prepare in advance for the changing seasons. Photoperiodism is used to cue the seasonal timing of reproduction, migration or dormancy in rotifers (Pourriot and Clémont, 1975), annelids (Fong and Pearse, 1992; Last and Olive, 1999, 2004; Schierwater and Hauenschild, 1990), molluscs (Joose, 1984; Ansart *et al*., 2001; Hommay *et al*., 2001), echinoderms (Halberg *et al*., 1987; Pearse *et al*., 1986), fish (Kemp, 1984; Bromage *et al*., 2001), amphibians (Laurila, *et al*. 2001), lizards (Fox and Dessauer, 1957; Licht, 1973; Cuellar and Cuellar, 1977), birds (Dawson, 2002; Dawson *et al*., 2001) and mammals (Goldman, 2001; Hofman, 2004) as well as arthropods where there exists a vast literature on insects (Danilevskii, 1965; Tauber *et al*., 1986; Danks, 1987; Saunders, 2002). From the insect citations, we make several generalizations about photoperiodism in temperate and more polar insect populations.

1. In general, day length provides the go/no-go cue for the direct timing of seasonal events or for the initiation of physiological, endocrinological and developmental cascades that once started are irrevocable or at least under natural conditions, usually not reversed before the completion of the seasonal event under selection. Day length may also modulate the rates of completion of these events through its interaction with food, temperature, and moisture. When there is an interaction between photoperiod and temperature, high temperatures tend to reinforce long-day effects and low temperatures short-day effects.

2. Because of the tilt of the earth relative to its plane of rotation about the sun, day length varies with both time of year and latitude (Fig. 1A). At the equator, the surface of the earth receives 12 hours of light per day (sunrise to sunset) all year long. As one proceeds north or south of the equator, the annual variation in day length becomes progressively more extreme, increasing from zero at the equator to 24 hours in the summer at latitudes greater than 67°. There are several consequences of these patterns for animals using photoperiod to time their seasonal activities. First, at tropical latitudes below about 15°, the annual change in day length is not sufficient to provide a reliable seasonal cue. Even so, a few insects are responsive to day lengths at latitudes as low as 9° but probably not within 5° of the equator (Norris,1965; Wolda and Denlinger, 1984; Denlinger, 1986). Second, in the temperate and polar zones above 30° latitude, wherein lies the greatest proportion of the earth's landmass, the annual change in day

length provides a strong and highly reliable seasonal cue over evolutionary time. The day length at a given latitude is the same today as it was 20,000 years ago and will be 20,000 years from now. This reliability is important. Insects cannot wait for often highly variable temperatures to change and for winter to arrive before entering dormancy or preparing for migration; rather, they use day length as an anticipatory cue to prepare for future seasonal change. Insects tend to enter diapause at a specific stage of their life cycle. During the developmental stage that is responsive to day length, the physiological decision is made to enter diapause or delay entry into diapause for another generation. The optimal time to enter diapause is then just over one full generation before the actual onset of winter (Taylor 1980), very often when the weather is warm and resources are still abundant. Hence, the adaptive significance of photoperiodism lies not only in the high reliability of day length as a cue but also in the anticipatory nature of using day length to switch off development and reproduction when the immediate environmental conditions are still favorable for these activities.

3. In temperate environments with warm, moist summers and harsh winters, especially in mid- or eastern continental climates, "long-day" arthropods usually enter an hibernal diapause that is initiated by short or shortening days. Timing is of the essence. An early entry into diapause results in the consumption of resources while temperatures are warm; a late entry into diapause runs the risk of encountering lethal winter conditions (Leather *et al.* 1993; Bradshaw *et al.*, 2004; Bergland *et al.*, 2005). Survivorship through winter or possessing resources available for development and reproduction the following spring are dependent on the timing of diapause the previous fall (Fig. 2). During the fall, insects are confronted with a go/no-go physiological decision, the consequences of which are not realized until some time in the future. Each individual has its own, genetically determined response to day length and, based on that response, makes the irrevocable physiological decision to diapause/continue development. Future environmental conditions (arrival of winter, duration of winter, resources available the next spring) then determine the fitness consequences of that decision. Since photoperiodism is the major physiological mechanism by which temperate insects time dormancy and migration in the fall (Danilevskii, 1965; Tauber *et al.*, 1986; Danks, 1987), fitness is dependent upon the proper response to day length and the target of selection in seasonal environments is the response to day length. The important point is that photoperiodism is an adaptation to length of the growing season, not to day length. As climates change over geography, so also does the optimal time to enter diapause and the optimal day length to use to switch from continuous development to diapause. Consequently, there is directional selection on the switching day length on a latitudinal scale but stabilizing selection on a local scale (Hard *et al.*, 1993a).

4. Photoperiod is generally important for timing the switch from continuous development to diapause in the fall, but diapause is usually terminated spontaneously or in response to a prolonged exposure to cold temperatures (chilling). In some cases, chilling may combine with food, moisture or day length to terminate diapause and, as we illustrate below (sec. X.5), modify rates of subsequent development.

X.2.3 Conclusion for Section 2

The tilt of the earth relative to its plane of rotation about the sun generates a climate at higher latitudes where summer seasons are favorable for growth, development and reproduction and alternate with winter seasons that are unfavorable for these activities. During winter, insects avoid or mitigate the exigencies of cold through migration and dormancy. Most insects are able

to use the length of day (photoperiodism) to anticipate and prepare in advance for the arrival of winter. Photoperiodism provides a highly reliable environmental cue that is stable through evolutionary time and provides the go/no-go signal that initiates a physiological and endocrinological cascade that commits an individual to a developmental pathway that is generally irrevocable within the lifetime of the insect or at least within a seasonal context.   At any given locality, there is an optimal time to enter diapause that is an adaptive compromise between avoiding winter cold and conserving resources for overwintering and for development and reproduction the following spring. Hence, the proper, genetically determined response to day length is an important determinant of fitness at temperate and more polar latitudes.   As the length of the growing season declines with increasing latitude or altitude, so also does the day length (critical photoperiod) that insects use to switch from active development to dormancy so that, while there is stabilizing selection on the critical photoperiod on a local scale, there is directional selection on a geographic scale. Once in diapause, the termination of diapause is often dependent upon accumulated chilling that may interact with moisture, food or day length. Along with ambient temperature, these same processes may affect the rates of post-diapause development in the spring. In the next section, we discuss the response of insects to climate change in the context of geographic patterns in day length and temperature.

X.3 Relative Roles of Day Length and Temperature in Biotic Response to Rapid Climate Change

X.3.1 Climate Change, Day Length and Temperature

Climate change does not alter the rotation of the earth about its axis or the rotation of the earth about the sun. Consequently, day length on any given day at any given latitude (Fig. 1A) does not change during periods of global warming. Climate warming at temperate and more polar latitudes does ameliorate the thermal environment, does alter the optimal timing of seasonal events and therefore does alter selection on the day length insects use to time these events (Bradshaw *et al*., 2004; Gomi *et al*., 2007). The phenotypic "fingerprint" of biotic response to climate change in animals is seen in the northward expansion of species' ranges, the earlier migration and reproduction in the spring, and the later migration or entrance into hibernation in the fall (Hughes, 2000; Peñuelas and Filella, 2001; Walther *et al.,* 2002; Root *et al.,* 2003; Parmesan and Yohe, 2003; Warren, 2006; Parmesan, 2006, 2007). These changes impose selection on *response* to day length. The extension of the growing season later into the fall selects for later entry into diapause and, hence, a shorter, more southern critical photoperiod. Indeed, evolutionary (genetic) responses to rapid climate change have resulted in shorter critical photoperiods (Bradshaw and Holzapfel, 2001a; Gomi *et al*., 2007). This genetic response to recent rapid climate change can take place over as short a time span as five years and is greater at higher latitudes where the climate is changing faster and selection is more intense than at lower latitudes (Bradshaw and Holzapfel, 2001a).

Recent rapid climate change has not occurred uniformly across latitudes and times of year. Climate warming has been proceeding faster with increasing latitude and in the winter than in the summer (IPCC, 2001, 2007). The net result is that winters are becoming milder, spring is arriving earlier, fall is arriving later, growing seasons are becoming longer and the duration and severity of winter cold is abating. The northern thermal years are becoming more like southern thermal years (Fig. 1B). Consequently, climate warming in the temperate and more northern

regions should be alleviating cold stress without imposing substantial heat stress (Bradshaw *et al.*, 2004; Bradshaw and Holzapfel, 2006). Indeed, over the next century, temperate and more northern insects are expected to achieve increasing, not decreasing fitness (r) due to the warmer temperatures alone and this effect increases with latitude (Deutsch *et al.,* 2008).

X.3.2 Photoperiodic vs. Thermal Adaptation in a Warming World

We used the pitcher-plant mosquito, *W. smithii* to determine the relative selective importance of temperature vs. photoperiodism during rapid climate warming (Bradshaw *et al.* 2004). *Wyeomyia smithii* lays its eggs and completes its entire pre-adult development only within the water-filled leaves of the purple pitcher plant, *Sarracenia purpurea*. The range of the mosquito follows that of its host plant from the Gulf of Mexico (30°N) to northern Canada from Labrador to northern Alberta (58°N). We simulated the natural, annual and daily progression of temperatures and day lengths of a more northern (Newfoundland, 50°N) and a more southern (New Jersey, 40°N) climate, using computer-driven, controlled-environment rooms. We then "transplanted" replicate northern populations to the more southern climate, representing a temperature change equivalent to 180-200 years of global warming at its present rate, and maintained controls in their native northern thermal year. We determined fitness as the year-long cohort replacement rate, integrating performance over all four seasons (Fig. 3A). All experiments were carried out in the leaves of intact pitcher plants using feeding schedules that mimicked prey capture by host plants in the field. The experiments pertinent to climate change involved three treatments:

● Northern populations in a *northern* thermal year programmed to enter and terminate diapause at the correct time for the northern thermal year (Fig. 3B).
● Northern populations in a *southern* thermal year programmed to enter and terminate diapause at the correct time for the southern thermal year (Fig. 3C).
● Northern populations in a southern thermal year allowed to express their northern, genetically determined response to day length in the foreign southern photic year (Fig. 3D).

The treatments in Figures 3B vs. 3C determine the comparative effects of temperature on fitness after genetically-determined responses to day length have been factored out. As shown in Figure 3E, northern populations achieved a greater fitness in the warmer, more southern thermal year (Fig. 3C) than in their native northern thermal year (Fig. 3B). These results are in accord with previous predictions that climate warming should alleviate, not exacerbate thermal stress on these northern populations (Bradshaw and Holzapfel, 2001a, 2006, 2008; Deutsch *et al*., 2008).

The treatments in Figures 3C vs. 3D determine the comparative effects of day length on fitness. As shown in Figure 3E, northern populations lost 88% of fitness when allowed to express their northern, genetically determined response to day length (Fig. 3D) as compared to their being programmed to enter and terminate diapause at the correct time of year (Fig. 3C). Hence, in a benign thermal environment, fitness was critically dependent upon possessing the correct, genetically-determined response to day length. *Simply put, the imposition of 180-200 years of climate warming promoted increased fitness among northern populations, but when these same populations encountered the wrong day length (Fig. 3D), they lost 88% of fitness.*

The cause of the differential fitness between the two photoperiod treatments in the benign thermal year (Figs. 3C vs. 3D) is apparent from the profiles of pupation during the same

simulated southern thermal year (Fig. 4). The genetically determined critical photoperiods of these northern populations range from 15-16 hours of light per day (Bradshaw *et al.*, 2003). At the start of the experiment, there was insufficient day length to stimulate direct development until day lengths exceeded the critical photoperiod and some larvae did not develop until the following spring. Had we simply transplanted populations in nature from the northern to the southern latitude (Fig. 3B vs. 3D), to determine the potential effects of climate warming on these northern populations and ignored day length, we would incorrectly have predicted that warmer temperatures associated with climate change would drive these populations to extinction. However, it was the inappropriate day length (Fig. 3C vs. 3D), not the warmer temperature (Fig. 3B vs. 3C) that resulted in the observed loss of fitness. The very important point here is that because the annual change in day length is fixed at each latitude (Fig. 1A), transplants from northern to southern localities in nature (e.g., Etterson and Shaw, 2001) cannot be used to evaluate the potential impact of climate warming because the effects of the novel photic environment will always confound the effects of temperature and result in an overestimation of the effects of climate change. However, the effects of changing thermal or moisture environments can be made correctly by transplants across longitudes (e.g. Fenster and Galloway, 2000), between altitudes at the same latitude as in the historic experiments of Clausen *et al.* (1940) or across time at the same locality by comparing contemporary with ancestral plants resurrected from appropriately stored seed in their common habitat (e.g., Franks *et al.,* 2007).

X.3.3 Conclusion for Section 3

In conclusion, when we compared directly the effects of temperature and photoperiod on the year-long cohort replacement rate that integrated fitness over all four seasons, possessing an inappropriate, genetically determined response to day length resulted in a drastic loss of fitness while climate warming tended to improve fitness of northern populations in accord with "warmer is better" (Frazier *et al.*, 2006; Deutsch, *et al.*, 2008). We have therefore concluded that in temperate and more northern climates, the primary target of selection during rapid climate change will be on the timing of seasonal events rather than on heat tolerance or thermal optima. Consequently, the primary genetic response of insect populations to rapid climate change will be photoperiodic rather than thermal adaptation; thermal adaptations would then be secondary adaptations that take place after populations are well established and have attained concordance with the local seasonal cycle. (Bradshaw *et al.*, 2000, 2004; Bradshaw and Holzapfel, 2001a, 2006, 2008). When considering the effects of climate change on fitness of populations *in situ* or of transplanted populations across latitudes, the effects of photoperiod need to be ruled out before any observed phenotypic or genetic shifts can be ascribed to the direct effects of temperature.

Mechanism does matter (Helmuth *et al.*, 2005; Biro *et al.*, 2007). Recent rapid climate warming at temperate latitudes has resulted in genetic shifts in photoperiodic response in insects, in migratory patterns or phenotypic plasticity in birds, in resource tracking by squirrels and in the frequency of genetic markers in flies (Bradshaw and Holzapfel, 2008). Most of the reaction to recent rapid climate change has focused on the important issue of curtailing or reversing CO_2 emissions (IPCC 2007). However, the climate will continue to warm for at least another century due to greenhouse gases and aerosols already present in the atmosphere (Warren, 2006; IPCC, 2007). It is therefore important to know how to mitigate the effects of future climate warming on biotic systems. In order to do so, we have to know the actual targets of selection in natural

populations of insects and the genetic ability of those populations to respond to selection, particularly in large animals with longer generation times and smaller population sizes than insects.

X.4 Genetic Architecture

X.4.1 Genetic Variability and Response to Selection

From the above, it is clear that climate warming is imposing selection for altered timing of seasonal activities and, hence, altered response to day length. Response to selection is dependent primarily on the strength of selection, the genetic variation on which that selection can act and on the extent to which selection in one dimension of the fitness landscape affects other dimensions of that landscape.

More formally, response to selection (R) is the product of the heritability of a trait (h^2) the strength of selection (S) applied to the trait: $R = h^2S$, where the heritability represents the genetic variation available for selection to act upon and can be thought of as a measure of the efficiency of response to selection. As discussed above, recent rapid climate change has resulted in longer growing seasons with earlier springs and later falls with concomitant selection on altered timing of seasonal activities. The magnitude of change in the length of the growing season then imposes a proportional selection pressure on the timing of seasonal activities and, consequently, response to day length. Since the rate of climate warming is increasing with latitude, so also is the rate of seasonal lengthening and the strength of selection (S) on response to day length. In *Wyeomyia smithii*, the heritability (h^2) of critical photoperiod also increases with latitude; hence, in hindsight, it is no surprise that the genetic shift towards shorter, more southern critical photoperiods (R) has increased with latitude over the last 30 years (Bradshaw and Holzapfel, 2001ab). In fact, this genetic shift can be detected over a time scale as short as five years, illustrating the great genetic potential of insects to keep pace with rapid climate change and the fact that phenotypic plasticity is not sufficient to account for biotic response to climate change.

Response to selection can be modified due to genetic correlations between the trait under selection and other traits that have an opposite effect on fitness. Essentially, if one imposes selection on a single trait and obtains a genetic response in a trait not under selection, then the two traits are genetically correlated. Genetic correlations can impede the simultaneous maximization of two fitness traits (Stearns, 1976, 1992; Rose, 1991; Roff, 1992, 2002). However, genetic correlations can facilitate as well as impede response to selection. In *Wyeomyia smithii,* generation time and critical photoperiod are positively genetically correlated (Hard *et al.,* 1993b), i.e., animals with shorter generation times are genetically predisposed to enter dormancy later in the year, in accord with theoretical predictions (Istock *et al.,* 1976; Taylor, 1980). The genetic correlations are coordinated rather than antagonistic with respect to the timing of seasonal development. Individuals with genetically shorter generations can effectively develop later in the season and still be in diapause by the time winter arrives. In addition, critical photoperiod forms part of a "diapause syndrome" where selection on shorter critical photoperiods results in fewer short days required to initiate diapause, a lower intensity of diapause and diapause in a later larval instar and vice-versa for selection on longer critical photoperiods (Campbell and Bradshaw, 1992). In this case, the genetic correlations within populations are the same as the genetic differences among populations and represents a gradient

from diapause-prone to diapause-averse genotypes, with the diapause-prone genotypes increasing with the severity and duration of winter. Response to selection on the timing of seasonal development is then reinforced rather than inhibited by the underlying genetic correlations.

X.4.2. Photoperiodism Genes?

Genetic correlations immediately beg the question as to the genes underlying photoperiodic response and how they interact. Heretofore, identifying genes responsible for photoperiodism has been strongly biased by assuming a causal connection between the circadian clock and the photoperiodic timer (Bünning, 1936; Withrow, 1969; Menaker, 1971; Pittendrigh, 1981; Pittendrigh and Takamura, 1993; Takeda and Skopik, 1997; Vaz Nunes and Saunders, 1999; Tauber and Kyriacou, 2001). Terrestrial animals living in temperate parts of the world encounter and exhibit two major periodicities: Daily periodicities include everything from daily behavioral activities to metabolic function, cell division, hunger, and sleep patterns. Primary control is by an internal, self-sustaining circadian clock that cycles or oscillates with a repeat period of about 24 hours. Circadian clocks orchestrate the daily transcription of hundreds of genes and are highly buffered against environmental variation and genetic background. The molecular genetic basis of circadian rhythmicity is well understood in insects, especially *Drosophila*. Seasonal periodicities include development, reproduction, migration and dormancy. Primary control is by response to day length. The molecular genetic basis of photoperiodism is not well understood in any animal, including *Drosophila*.

A causal relationship between the circadian clock and photoperiodism would mean that one or more genes are mediating both processes, i.e., their functional connection is due to pleiotropy. In this case, pleiotropy would be bi-directional: selection on one trait would generate a correlated response in the other trait, leading to potential tradeoffs between fitness-related traits (Rose, 1991; Roff, 1992; Stearns, 1992). The circadian clock orchestrates the daily temporal coordination of hundreds of genes in Drosophila (Claridge-Chang *et al*., 2001; McDonald and Rosbash, 2001; Ceriani *et al*., 2002; Duffield *et al*., 2003) and is insulated against environmental perturbation, especially temperature, is re-set every day and persists under constant conditions (Pittendrigh 1960, 1981). By contrast, photoperiodic time measurement enables organisms to anticipate and prepare in advance for future seasonal changes in their environment, and the correct, climate-specific photoperiodic response is essential for maintaining fitness in temperate seasonal environments (Bradshaw *et al*., 2004). Photoperiodic response is genetically variable, evolutionarily flexible, is often affected by food temperature or moisture and usually signals a go/no-go switch that is usually irreversible and does not repeat under constant conditions. If circadian rhythmicity and the evolutionary modification of photoperiodic time measurement were causally connected through pleiotropy, then rapid evolution of photoperiodic time measurement by invading species (Hoy, 1978; Tauber *et al*., 1986, pp. 238-245; Fochs *et al*., 1994; Lounibos *et al*., 2003) or in response to rapid climate change (Bradshaw and Holzapfel, 2001a; Gomi *et al*., 2007) would necessarily involve significant adjustments in the precise daily coordination of important metabolic events. The daily clock and the seasonal timer serve two, separate adaptive functions and are affected by different suites of environmental inputs (Danks, 2005).

In *Drosophila littoralis*, critical photoperiod (photoperiodic response) and period of the eclosion rhythm (circadian response) are both correlated with latitude but this correlation is due

to independent evolution of both traits and the apparent genetic correlation between them is due to linkage and not pleiotropy (Lankinen, 1986ab; Lankinen and Forsman, 2006). In North American *Wyeomyia smithii*, critical photoperiod is positively correlated with latitude and altitude but is not correlated with either the period or amplitude of the circadian clock (Bradshaw *et al*., 2006). Hence, in both *D. littoralis* and *W. smithii*, the photoperiodic timer has evolved independently over the climatic gradients of their respective continents. It is difficult to argue a causal relationship between the circadian clock and the evolution of photoperiodic response when the two are not correlated over strong selection gradients through evolutionary time.

At the molecular level, all investigations of which we are aware, including some of our own, have treated circadian rhythm genes as candidate loci for photoperiod genes. The search for the molecular basis of photoperiodism through studies of specific circadian clock genes, especially in Diptera (Saunders, 1990; Saunders *et al*., 1989; Mathias *et al*., 2005, 2007; Goto *et al*., 2006; Tauber *et al*., 2007; Sandrelli *et al*., 2007; Stehlik *et al*., 2008) has revealed more about circadian rhythm genes themselves, but the genetic mechanisms underlying the evolution of photoperiodic response remain elusive (Bradshaw and Holzapfel, 2007, 2008). At the time of this writing, there has not been a single photoperiod gene identified in a natural population of any animal. Future searches for the mechanistic basis of photoperiodism and its evolution should therefore focus on forward genetic approaches (working from phenotype to genotype) such as fine-scale mapping of genes on chromosomes or microarrays showing differential gene expression (Mathias *et al*., 2007; Benfey and Mitchell-Olds, 2008; Stinchcombe and Hoekstra, 2008) that are unbiased by the assumption of a causal connection with the circadian clock (Bradshaw and Holzapfel, 2007; Tauber and Kyriacou, 2008). Towards this end, we have developed the first QTL map of photoperiodic response in any animal (Fig. 5). This map shows that there are some 6-9 regions of the *Wyeomyia smithii* genome involved in the evolution of response to day length. This map confirms prior quantitative genetic results that the evolution of critical photoperiod in *W. smithii* is a complex trait involving additive, dominance and epistatic effects of alleles within and between multiple loci and a genetic correlation between critical photoperiod and stage of diapause (Hard *et al*., 1993b; Campbell and Bradshaw, 1992; Lair *et al*., 1997; Bradshaw and Holzapfel, 2001b; Bradshaw *et al*., 2005). Concurrently, we were able to develop a QTL map for stage of diapause. Most intriguingly, QTL 8 (Fig. 5) coincides with a QTL for stage of diapause and is also centered over two genes involved in continuous development (ecdysteroid receptors). Hence, this region of the genome may be responsible for coordinating the external environment (day length), continuous development and diapause.

X.4.3 Conclusion for Section 4

Insect populations harbor sufficient genetic variability in their response to day length to track selection on the timing of seasonal development imposed by recent rapid climate change. Genetic correlations involving critical photoperiod are coordinated to form a continuum between diapause-prone to diapause-averse genetic syndromes so that response to selection on the timing of seasonal development and diapause is reinforced rather than inhibited by the underlying genetic correlations. The search for genes responsible for photoperiodism at the molecular level have provided tantalizing but elusive connections between the daily circadian clock and evolution of the seasonal photoperiodic timer. Apart from the QTL map in *W. smithii*, all approaches at the molecular level have treated circadian genes as candidate loci for genes involved in photoperiodism. Forward genetic approaches starting with photoperiodic phenotypes

themselves and working to the causative genes themselves are more likely to reveal the genes actually responsible for photoperiodism and its evolutionary response to selection along seasonal gradients or to rapid climate change. Finally, the coincidence in a single region of *W. smithii*'s genome of QTL affecting photoperiodism and diapause as well as genes involved in continuous development indicate that this region may form a co-adapted gene complex responsible for coordinating the external environment, development and diapause.

X.5 Response to climate change when it's actually warming

The very term "global warming" invites the assumption that a warmer climate means heat when it's hot, i.e., during the summer, and that we can understand the main effects of climate change by determining the phenotypic and genetic limits and evolvability of heat tolerance and thermal optima. But, as we have pointed out, surface temperatures on the earth are increasing faster in the winter than in the summer (IPCC, 2001, 2007) and, at temperate and higher latitudes, climate warming is expected to increase, not decrease insect fitness (Bradshaw *et al.*, 2004; Bradshaw and Holzapfel, 2006; Deutsch *et al.*, 2008). The question then remains as to the consequences of climate warming at the time of year when temperature is now increasing at its maximum rate, i.e., during the winter when insects are in diapause.

A wide variety of temperate arthropods use day length to program the initiation of diapause but, once in diapause, most insects become refractory to day length and rely on other environmental factors such as moisture, food and, most often, chilling to terminate diapause (Andrewartha, 1952; Lees, 1955; Tauber *et al.*, 1986; Leather *et al.*, 1993; Koštál, 2006). The termination of diapause by chilling during the winter leaves insects in a quiescent state where development is usually constrained by the direct effects of temperature. Once diapause is terminated, synchronization of vernal development is then achieved passively due to the cumulative effects of developing in a thermal environment of rising temperatures (Bradshaw, 1973; Danks, 1987). The reduction in intensity and ultimate termination of diapause by chilling is a temperature-dependent process. The effect of temperature on the termination of diapause is much like the effect of temperature on continuous development. There is an optimal temperature at which the intensity of diapause decreases at the fastest rate and lower and higher temperatures at which the intensity of diapause does not change. (Lees, 1955; Bradshaw and Holzapfel, 1977; Tauber *et al.*, 1986; Danks, 1987; Leather *et al.*, 1995).

While most insects become refractory to day length while in diapause, many do not. In those that do not, short days tend to maintain and long days tend to terminate diapause. However, chilling may still proceed so that the termination of diapause and post-diapause development combine into a dynamic interaction between chilling time and day length (Fig. 6). Vernal development is then a function of the degree by which winter temperature departs from the chilling optimum since departure from this optimum can determine the intensity of diapause, the date on which diapause terminates, and the response to day length. Assuming that winter temperatures are near the thermal optimum for chilling, the intensity and termination of diapause and vernal development would normally proceed along the diagonals from lower left to upper right in Figure 6, accelerating the termination of diapause (Fig. 6a) and potential rates of post-chilling development (Fig. 6b). As climate change has resulted in warmer winters and earlier springs, most insects have advanced the timing of their vernal development; however, there are a few butterflies whose vernal development has not changed or has been delayed, despite the warmer spring (Roy and Sparks, 2000; Forister and Shapiro, 2003). We propose that, in these

cases, winter temperatures may have warmed enough to void or abate the effects of chilling and therefore have left those insects still in diapause at the end of winter and/or requiring longer days and more time to develop despite the warmer spring. This effect is already seen in trees where the warmer winters do not provide sufficient cold to "satisfy" the requirement for vernalization (Schwartz *et al*., 2006).

X.5.2 Conclusion for Section 5

Although much of the research related to climate warming has investigated the phenotypic range, heritability and evolvability of heat tolerance and thermal optima during the growing season, the most rapid rate of climate warming is occurring during the dormant, winter season. Warmer winter temperatures that retard the chilling process and, hence, the termination of diapause may provide the reason why, despite earlier, warmer springs, some species have not advanced or have even delayed vernal development. Understanding the impact of recent rapid climate change on insect populations is going to involve understanding the effects of warmer winter temperatures on the maintenance and termination of diapause and on post-diapause development and reproduction in the spring.

X.6. Unexplored implications of climate warming and gene flow.

X.6.1. Range Expansion and the Depletion of Heterozygosity

The two major overt patterns of biotic response to climate change in the temperate zone have been the altered timing of seasonal events and the poleward expansion of species' ranges (X.3, above). In the latter case, individuals are expanding their ranges into a vacant niche, i.e., unoccupied by members of their own species. In the northern hemisphere, this migratory pattern is not new, but has been taking place since recession of the Laurentide Ice Sheet over the last 8,000 – 20,000 years. As immigrants from a more southern area found new, more northern populations, they take with them only a fraction of the genetic variation of the ancestral source population. Isolation and drift then leave the new population with lower heterozygosity than the source population. The new population grows and ultimately produces emigrants who found even newer populations but bring with them an even smaller fraction of the alleles from the original source population. A northward succession of founder events then creates a latitudinal gradient of decreasing heterozygosity in plants (Schwaegerle and Schaal, 1979; Cwynar and MacDonald, 1987), earth-bound animals (Highton and Webster, 1976; Bellemin *et al*., 1978; Green *et al*., 1996) and insects (Stone and Sunnuck, 1993; Armbruster *et al*., 1998). The question remains as to why heterozygosity remains depleted in the new populations and is not replenished from more southern populations as the latter continue to grow and flourish. We propose that the answer may relate (1) to the distinction between the original founder event, which took place into a vacant niche, previously unoccupied by conspecifics, and subsequent immigration of individuals, which takes place into a niche already occupied by conspecifics and (2) to the difference in temperature-dependent fitness between the southern, more warm-adapted immigrants and the northern, more cold-adapted residents.

X.6.2. Fitness at a Single Locality

Based on both theoretical and empirical observations (Frazier *et al.*, 2006), fitness increases exponentially with temperature up to maximum fitness (r_{max}) at the thermal optimum (T_{opt}) (Fig. 7A) and declines precipitously to zero at higher temperatures (T_{max}). Fitness for any temperature T (W_T) can then be described (Deutsch *et al.*, 2008) from a Gaussian function for the rising portion of the curve, from a quadratic function for the falling portion of the curve, from the width of the curve (performance breadth) in units of phenotypic standard deviation (σ) and from r_{max}, fitness at T_{opt}:

$$W_T = r_{max}\left[\exp\left\{-\left(\frac{T - T_{opt}}{2\sigma}\right)^2\right\}\right] \text{for} T \leq T$$

$$W_T = r_{max}\left\{1 - \left(\frac{T - T_{opt}}{T_{opt} - T_{max}}\right)^2\right\} \text{for} T > T_{opt}$$

Frazier *et al*. (2006) make several important empirical generalizations. First, cold-adapted insects reach their upper limits of tolerance (T_{max}) at lower temperatures than do warm-adapted populations. Second, cold-adapted insects achieve lower fitness (r_{max}) at their thermal optimum (T_{opt}) than warm-adapted insects do at their thermal optimum. Third, higher latitude populations experience a wider range of daily and annual temperatures than do low latitude populations. Consequently, northern, more cold-adapted populations tend to have a wider range of thermal tolerance (performance breadth, σ).

X.6.3. Relative Fitness of Immigrants and Residents

Figure 7B compares the fitness functions between two conspecific populations, a more northern, cold-adapted population (solid line) and a more southern, warm-adapted population (dashed line) in close enough proximity to so that there could be gene flow between them. In accord with Frazier *et al.*'s (2006) conclusions, the northern population (1) has a lower thermal maximum, (2) a lower thermal optimum at which it achieves lower fitness and (3) a wider range of thermal tolerance.   Note that, as long as the warming temperatures are below the optimal temperature (T_{opt}) for the northern resident population, southern immigrants will always be at a fitness disadvantage. During periods of rapid climate warming (ambient temperature shifts from the solid to the dashed vertical line), gene flow from southern, more warm-adapted populations into northern, more cold-adapted populations will continued to be inhibited so long as the increase in summer temperature does not exceed T_{opt} of the more northern population. To put this last conclusion into perspective, the increase in temperature over the next 100 years is not expected to exceed T_{opt} of temperate and more northern insects in general (Deutsch *et al.*, 2008). If summer warming as a consequence of rapid climate change imposes selection on the thermal optimum or on heat tolerance, evolutionary response to that selection at temperate and more northern latitudes is going to take place primarily by changes in gene frequencies in extant populations rather than by gene flow from more southern populations.

These conclusions are eminently amenable to experimental testing. Frazier *et al.* (2006) list a large number of insects with a wide range of thermal performance curves relating fitness to temperature. If two populations in the same species can be identified that satisfy the conditions

in Figure 7B, it is then straightforward to determine whether an immigrant from a more southern population introduced into a more northern population is able to invade that population successfully at temperatures below T_{opt} for the more northern population. The "success" could be evaluated from molecular markers that are now feasible for non-model organisms.

X.6.4. Conclusion for Section 6

Range expansion accompanying recent rapid climate change has often involved migrant populations entering into a vacant niche, unoccupied by conspecifics. However, the situation is very different for gene flow within the range of existing conspecific populations in temperate and more northern latitudes. If northern populations are more cold-adapted and have a wider range of thermal tolerance, immigrants from a more southern, warm-adapted population will be at a consistent fitness disadvantage compared to the more northern population even if the southern population has a warmer thermal optimum and higher tolerance of heat. This lower fitness of immigrants may explain why the decrease in genetic variability with increasing latitude in post-glacial populations persists despite the opportunity for immigration from more southern, more genetically variable source populations.

X.7 When You Aren't Always Photoperiodic – the Special Case of *Drosophila*

X.7.1 Photoperiodism in *Drosophila*

"Although *D. melanogaster* with its unrivaled genetic background has provided a foundation for uncovering the molecular basis of the circadian mechanism… it is probably less useful as a model for photoperiodism" (Saunders, 2002). *Drosophila melanogaster* likely originated in central Africa and has a very recent history in the temperate zone. In Europe, where it has invaded during the last ~10,000-15,000 years ago after the last glaciation (Lachaise *et al*. 1988, Lachaise and Silvain, 2004; Baudry *et al*., 2004), *D. melanogaster* is photoperiodic. However, European populations exhibit a linear, graded response to day length over a range of 8-18 hours of light per day (Tauber *et al*., 2007), rather than a steep, sigmoid response that is characteristic of most insect populations (Danilevskii, 1965; Danks 1987; Saunders, 2002). In these European populations, photoperiod is acting more like a continuous modulator of development, rather than a physiological go/no-go trigger of diapause. In North America where *D. melanogaster* likely originated from Africa within the last 300-400 years (Lachaise *et al*. 1988; David and Capy, 1988), only the Canton-S strain of *D. melanogaster* is unambiguously photoperiodic and only over a very restricted range of temperatures; all other strains tested are dubiously photoperiodic or clearly not photoperiodic (Saunders and Gilbert, 1990; Tatar et al. 2001). Even in Canton-S, the narrow range of temperature over which they are photoperiodic suggests that photoperiod would have little influence on the timing of diapause in natural populations. At a constant low temperature, North American *D. melanogaster* from Florida to Maine show a clear linear cline in incidence of diapause (Schmidt *et al*., 2005). It would therefore appear that the primary determinant of diapause in *D. melanogaster* is low temperature, consistent with the control of diapause by temperature in tropical and subtropical insects in general (Denlinger, 1986).

Despite a lack of a strong photoperiodically induced diapause, non-photoperiodic *Drosophila* exhibit seasonal and geographic variation in allozymes and chromosomal inversions

that have shifted in their frequency over the last 40 years in a manner consistent with a genetic response to recent climate change.

Dobzhansky (1948) observed that chromosomal inversions in Californian populations of *Drosophila pseudoobscura* changed with seasonality such that the frequency of certain inversions increased during the summer and decreased during the winter. At the same time, the frequency of the winter inversions increased with latitude and altitude. Hence, the inversions are under seasonal selection.

Seasonal cycling and a latitudinal cline in inversion frequencies also occur in European *D. subobscura*. With increased climate warming the frequency of the northern inversion declined over 16 years in Spanish populations (Rodríguez-Trelles and Rodríguez, 1998). During the 1970s, *D. subobscura* has also independently invaded western North and South America and on both continents established a latitudinal cline in inversion frequencies reflecting the ancestral cline in Europe (Ayala *et al.,* 1989; Prevosti *et al.,* 1988). After 24 years between samplings, climates had warmed significantly in 22 of 26 populations and, concomitantly, in 21 of these populations, there has been a shift towards inversions characteristic of low latitudes on all three continents (Balanyá *et al.,* 2006). In central and eastern North America, there has also been an increasing frequency of southern inversions in *D. robusta* that parallels increasing local minimum air temperatures (Levitan, 2003; Levitan and Etges, 2005). Finally, over a 20-year period in eastern Australia, there has been a southern shift in both inversion and allozyme frequencies in populations of *D. melanogaster* (Umina *et al*., 2005). Hence, at the local, continental, and global scales, there has been a shift towards southern or low-elevation inversions or allozyme frequencies that parallels recent climate warming across four species of *Drosophila* and four continents.

The increase in the "summer" or "southern" inversions in *Drosophila* means that in populations as a whole, there has been a genetic shift towards genotypes associated with warmer climate. However, it is not known whether this genetic shift has been due to hotter summer temperatures, per se, or due to a longer warm and a shorter cool season during which the alternative selective forces can act. In the first case, we would expect there to be greater thermal tolerance or a higher thermal optimum for the summer or winter inversions in contemporary than ancestral populations. In the second case, we would expect there to be no change in thermal tolerance or optima associated with specific inversions and would conclude that the shift in inversion frequency has been due to the length of the growing season rather than higher temperatures, per se (Rodríguez-Trelles and Rodríguez, 2007).   In addition, there is a latitudinal cline in genetic tendency to diapause among populations of *D. melanogaster* (Schmidt *et al*., 2005; Tauber *et al*., 2007) and a Dutch population of *D. melanogaster* has a longer critical photoperiod than Italian populations (Tauber *et al.,* 2007). The changes in inversion frequencies may therefore also be related to selection for reduced incidence of diapause or a shorter critical photoperiod. In short, we know that southern inversion and allozyme frequencies are positively correlated with recent, rapid climate change but not whether this pattern results from selection on thermal optima, thermal tolerance or season length.

X.7.2 Conclusion for Section 7

Different populations in different species of *Drosophila* that are not known to be photoperiodic undergo seasonal cycling of allozyme and inversion frequencies. The "summer" allozymes or inversion types also decrease with increasing latitude, indicating a high genetic

sensitivity of *Drosophila* populations to seasonal selection. During recent, rapid climate change, there has been a pervasive tendency for "summer" or "southern" allozyme or inversion frequencies to increase in extant populations, indicating a persistent genetic change in these populations. These shifting frequencies could result from selection on thermal optima, heat tolerance, season length or incidence of diapause. Thermal rather than photoperiodic control of diapause appears to be the norm for *D. melanogaster, D. subobscura* and *D. robusta* under natural conditions. These species are the most likely candidates for demonstrating an evolutionary (genetic) response in thermal tolerance or thermal optima to rapid climate change.

X.8 Summary

Global warming is proceeding fastest during the winter rather than in the summer and the rate of climate warming is fastest at more polar latitudes where the gradient in winter cold is steepest, thereby alleviating cold stress without imposing appreciable summer heat stress and improving fitness of temperate and polar ectotherms. Earlier springs and later falls have resulted in longer growing seasons and the major biotic responses to recent rapid climate change at temperate` and polar latitudes have been altered timing of seasonal activities and a poleward extension of species' ranges. The target of selection is then on traits related to the seasonal activities of animals, not thermal adaptations. There are now examples of genetic shifts in the seasonal timing of development and reproduction in plants, insects, birds and mammals in response to recent rapid climate warming in the temperate zone; but, to our knowledge, there are no genetically based examples of any animal increasing its thermal optimum or tolerance of higher summer temperatures. A wide variety of animals from rotifers to rodents use day length (photoperiodism) to time their seasonal activities and a genetic change in critical photoperiod during recent rapid climate change has been demonstrated in at least two insects.

In accord with these considerations, when northern populations of *Wyeomyia smithii* are transplanted to a warmer more southern thermal year, fitness increases; however, when the northern populations are exposed to the more southern day lengths as well as the more southern thermal year, fitness declines by 88%. These results mean first that exposure to the incorrect day length, not the warmer temperatures, resulted in the dramatic loss of fitness. Second, to assess the potential impact of climate change on populations, transplantation from northern to southern localities in nature will always confound the adverse effects of genetically programmed responses to the foreign day length with the effects of a warmer climate, thereby overestimating the effects of temperature. Third, the effects of photoperiod need to be ruled out before any observed phenotypic or genetic changes associated with climate change can be ascribed to the direct effects of warmer temperature. Mitigating the effects of continued climate change will depend crucially on knowing the actual targets of selection in natural insect populations and the ability of those populations to respond to selection. Insects generally consist of large populations with short generation times and high heritabilities for response to day length. The genetic correlations related to photoperiodism can be coordinated rather than antagonistic so that response to selection on the timing of seasonal development is reinforced rather than inhibited by the underlying genetic architecture.

The search for genes involved in photoperiodism at the molecular level has largely used circadian clock genes as candidate loci with little definitive results. Forward genetic approaches such as fine scale quantitative trait loci mapping and/or cDNA microarrays to assess expressed genes are more likely to reveal genes involved in photoperiodism and its adaptive

evolution over climatic gradients and in response to rapid climate change. The sole extant QTL map for photoperiodism in any animal indicates 6-9 QTL and a region of the genome containing genes affecting critical photoperiod, stage of diapause and receptors for hormones involved in continuous development suggests spatial coordination of genes involved in interpreting the external environment and making the go/no-go switch between continuous development and diapause.

Temperate and more polar climates are warming primarily during the winter and not the summer, but little consideration has been given to the potential effects of climate warming on the progression of diapause during the winter months. We show that the intensity of diapause, rates of response to day length and the completion of post-diapause development are all affected by the duration of winter cold (chilling). Recent, warmer winters may then not provide sufficient cold to reduce the intensity of diapause and may thereby explain why, despite earlier, warmer springs, the timing of vernal development has not changed or may even be retarded for some insects during recent climate change.

Although species are expanding their ranges poleward, this expansion primarily involves immigration into vacant niches. However, in those cases when the northern regions are currently occupied by conspecifics that are already cold-adapted, immigrants from a southern population will consistently be at a fitness disadvantage, based on strictly thermal considerations. This lower fitness of conspecific immigrants may explain the persistence of low genetic variability in post-glacial populations despite the opportunity for southern, genetically variable source populations to contribute to these impoverished gene pools, despite thousands of years of opportunity to do so.

Finally, we consider the various *Drosophila* that are not photoperiodic, weakly photoperiodic or photoperiodic over only a narrow range of temperatures. With the very short generation times and large population sizes, *Drosophila* populations change genetically over the time span of the four seasons or over a few years of climate change and are therefore highly responsive to seasonal selection. Whether this responsiveness results form selection on thermal optimum, thermal tolerance, season length or incidence of diapause is yet to be determined.

X.5.2 Some Predictions and Implications

1. As more southern species invade more northern latitudes, biodiversity at northern latitudes should increase. This prediction is consistent with the observation that biodiversity increases towards the tropics, so that, as more northern latitudes become warmer, species richness should increase.

2. Since biotic interactions tend to be more important than physical limiting factors in benign environments and since predators tend to be larger, have longer generations and smaller population sizes than their prey, we can expect that competitive interactions among insect herbivores and that the impact of these herbivores on plants will intensify with increased climate warming. These relationships will alter the timing and intensity of biotic interactions, leading to altered community structure and ecological "surprises," communities that lack contemporary analogs (Williams and Jackson, 2007).

3. Along with other insects, vectors of disease will expand their ranges northwards and, as the favorable season increases, there will be more time for pathogens to complete the extrinsic

incubation cycle in their insect hosts. Consequently, there will be an increased diversity of vectors with greater competence to transmit pathogens among wildlife, livestock and humans.

4. The genes controlling photoperiodism and, hence, seasonal timing of many insects will consist of a network of interacting genes that coordinate the interpretation of the environment with continuous development and diapause.

5. Identification of photoperiod gene networks and their interaction with hormonal control of development and diapause may provide a means for controlling the expected increase in the abundance of agricultural pests and disease vectors, much as the use of juvenile hormone analogues have provided "third generation" pesticides for the control of mosquitoes.

Acknowledgments

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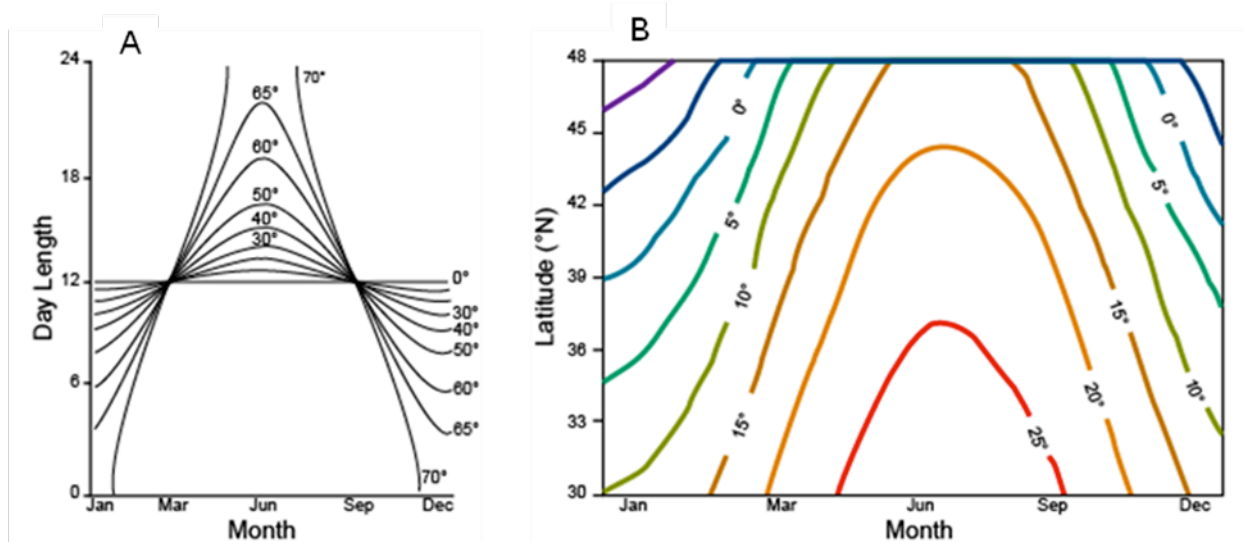
Figure X.1. Geographic and seasonal variation in day length and temperature. (A) Seasonal patterns in day length (sunrise to sunset) at different latitudes (°N) in the Northern Hemisphere (Danilevskii, 1965). Day length at temperate and polar latitudes predicts future seasons more reliably than any other environmental cue. (B) Isotherms for mean monthly temperature in central and eastern North America (Bradshaw et al. 2004). The latitudinal variation in climate is less a matter of summer warmth (June isotherms are far apart) than winter cold (January isotherms are close together) and northern populations experience a shorter growing season than southern populations. Hence, changes in season length and the timing of spring and fall activities have a greater effect on animal populations than do the direct effects of temperature.
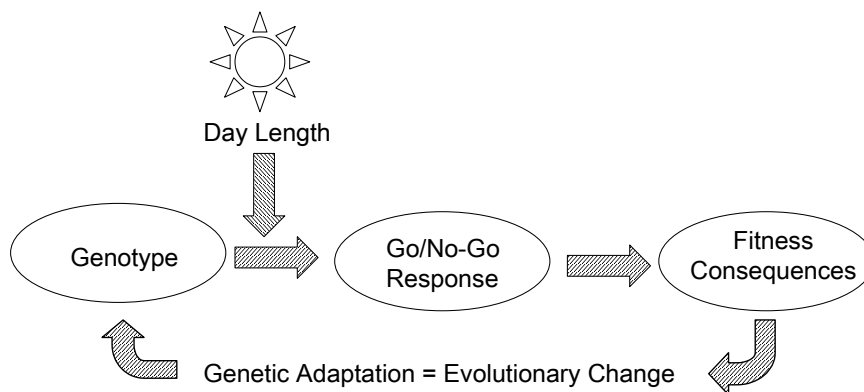

Figure X.2. Selection on photoperiodic response. The photoperiodically mediated go/no-go physiological decision to diapause or to continue developing is based on the combination of an individual's genotype and the day length it sees. The resultant timing of diapause then has fitness consequences that impose selection on genotypes that respond to different day lengths. Since, insects can enter diapause too early or too late in the fall, there is stabilizing selection on the optimal response to day length.
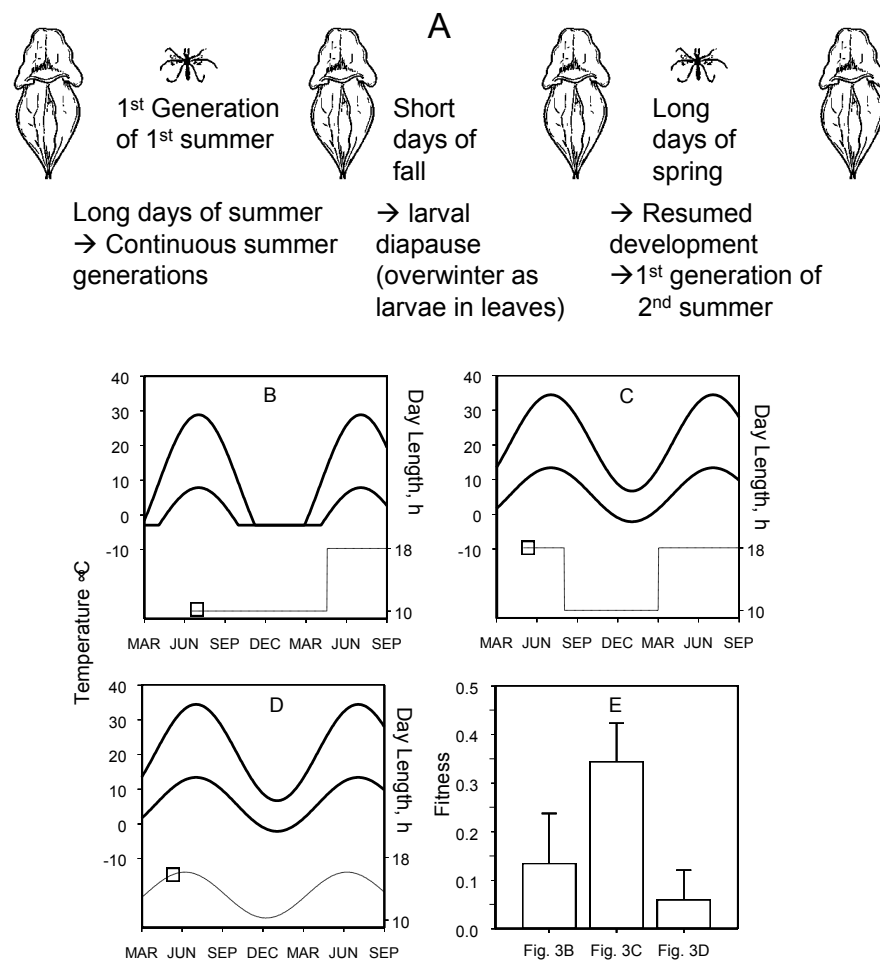
Figure X.3. Determination of fitness integrated through all four seasons. **(A)** Determination of the year-long cohort replacement rate: Ry = (number of first instars hatching from the overwintering cohort as the first generation of the second summer) ÷ (number of first instars introduced into the pitcher plants leaves as the first generation of the first summer). **(B-D)** Simulated thermal years of 50 and 40°N showing maximum and minimum daily temperatures (upper and lower solid lines, respectively) and day length (dashed line). Experimental cohorts are started weekly during a one-month period indicated by the box □ and continue through the spring and early summer of the next year. An 18-hr day length is a long day for all populations regardless of their latitudinally-specific genetically-programmed response to day length and, hence, stimulates development in all populations. Likewise, a 10-hr day length is a short day and initiates and maintains diapause in all populations regardless of their latitudinally-specific, genetically-programmed response to day length. In this way, we are able to factor out genetic differences in response to day length and to induce and terminate diapause at the latitudinally-appropriate time of year. **(E)** Fitness achieved by northern populations exposed to the three environments in Fig. X.3B-D. A comparison between Fig. 3B vs. 3C tests directly for thermal adaptation when response to day length is factored out; a comparison between Fig. 3C vs. 3D tests directly for photoperiodic adaptation when temperature is held constant. Fitness is plotted as $Log(Ry + 1)$ + one standard error.
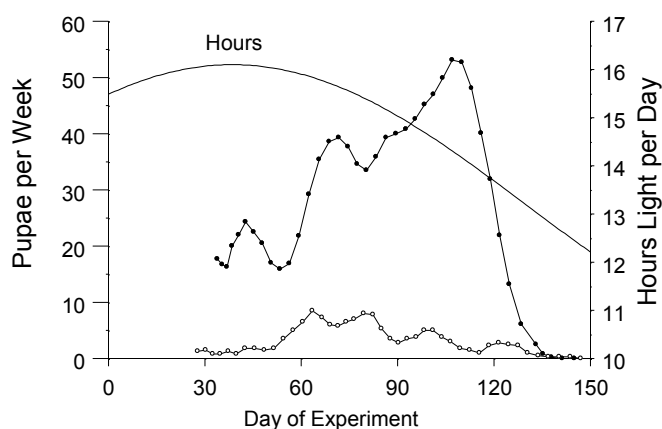
Figure X.4. Seasonal trajectory of pupation by northern populations subjected to a benign southern thermal year and either (●) programmed to enter and terminate diapause at the correct time of year as in Figure. X.3C or (○) allowed to express their northern, genetically determined response to day length in a foreign southern photic year as in Figure. X.3D.
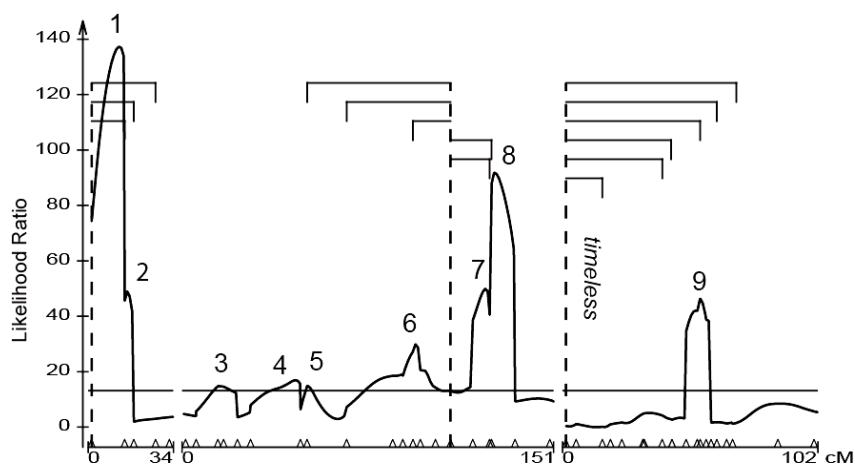
Figure X.5. Quantitative trait loci (QTL) map for critical photoperiod in *Wyeomyia smithii* based on the F_2 critical photoperiods of a single-pair cross between a northern and a southern population (Mathias *et al*., 2007). AFLP and gene markers are indicated by open triangles. Peaks with likelihood scores above the horizontal black line are indicative of a likely QTL. The two breaks in the horizontal black line, indicate the three chromosomes. Digenic epistatic interactions between markers are shown as brackets subtended by a dashed line.
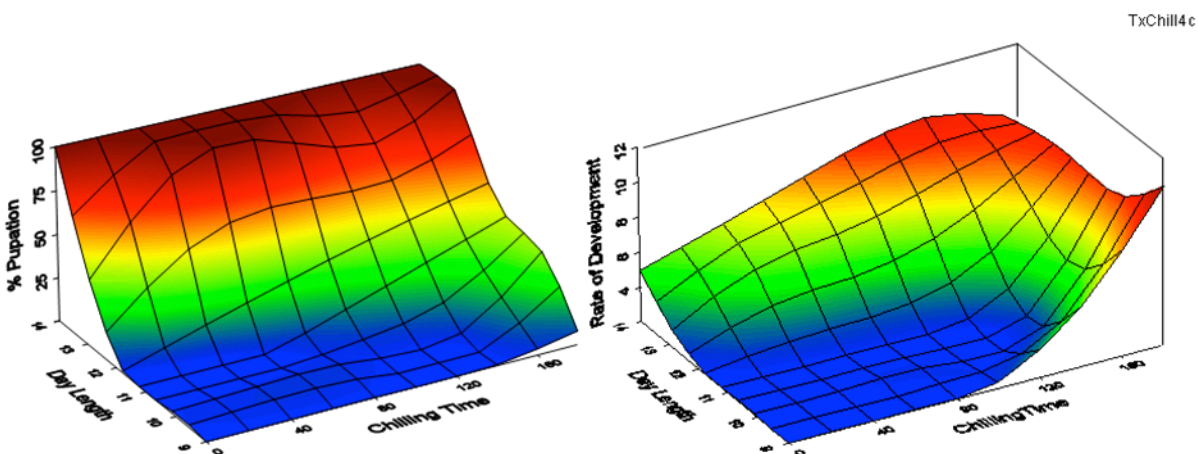
Fig. X.6. Effect of day length and chilling at 7°C on subsequent termination of larval diapause and rate of development at 21°C in the tree-hole mosquito, *Toxorhynchites rutilus* from 41°N (Bradshaw and Holzapfel, 1977). (A) Percent pupation within 50 days post-chilling at 21°C (R^2 = 0.86). (B) Rate of development (100/days to pupation) among larvae pupating within 50 days post-chilling at 21°C (R^2 = 0.84).
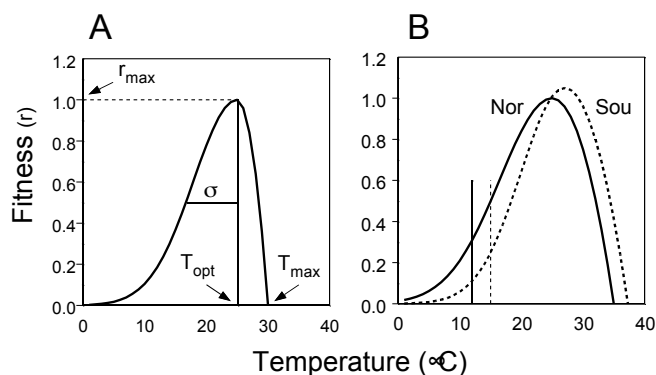
Figure X.7. Effect of temperature on ectotherm fitness. (A) The performance curves are derived from empirically estimated optimal temperatures (T_{opt}) at which maximum fitness occurs (r_{max}), the upper thermal threshold (T_{max}) and performance breadth (σ, the standard deviation of the rising logistic function) (Deutsch et al., 2008). (B) Simulated comparison of temperature-dependent fitness in a northern, cold-adapted population (solid line) and a slightly more southern, warm-adapted population (dashed line). The vertical lines indicate temperature before (solid) and after (dashed) a period of climate warming. (see text for equations; North: T_{opt} = 25; r_{max} = 1.0; T_{max} = 35; σ = 5; South: T_{opt} = 27; r_{max} = 1.1; T_{max} = 37; σ = 6).