Slaves to the Eyring Equation?

Temperature dependence of life-history characters in developing ectotherms

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Abstract

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This thesis investigates to what extent the thermodynamics of biological rates constrains the thermal adaptation of developing ectotherms. The biophysical Sharpe – Schoolfield model is applied to explain the temperature dependence of body size in ectotherms, to predict the temperature tolerance limits in developing ectotherms and to predict patterns of thermal adaptation within and among species. If the Sharpe – Schoolfield equation is applied to model the temperature dependence of growth and differentiation rate separately, then the temperature dependence of size at maturity follows from the interaction between these processes. Recent studies have shown that this approach provides an explanatory framework for all ectotherms, which obey the Temperature – size Rule, the observation that ectotherms at high temperatures grow and develop faster to a smaller size at maturity compared to low temperatures, but also to the exceptions of this rule.

The Sharpe – Schoolfield equation basically consist of two parts: the numerator, which is formed by the Eyring equation, models the exponential increase of reaction rates with temperature based on reaction kinetics, and the denominator, which describes the reversible temperature-induced inactivation of enzymes. If the denominator is applied to a genetic control system of the cell cycle, it can be shown that the temperature tolerance limits are accurately predicted in range of insect species. It is argued that reversible temperature-induced inactivation of regulatory components of the cell cycle mimics the dosage change during the cell cycle. The Eyring equation is also successfully applied to cross-species comparisons of thermal adaptation in a large group of related frogs and toads. The recently developed model of Universal Temperature Dependence is critically discussed and it is argued that the predictions are partly based on incorrect assumptions and biased use of literature data. Furthermore, the supposed invariant biophysical parameters may vary in response to thermal adaptation.

When ectotherms adapt to lower temperatures (horizontal shift) a correlated response occurs of a wider thermal range (specialist – generalist shift), a smaller slope (sensitivity shift) and lower activity (vertical shift). This correlated response is mainly determined by the Eyring equation. The enzyme activity – stability tradeoff is the most important thermodynamic constraint and limits the viable development of most ectotherms to a relative small thermal tolerance range of approximately 20 °C. It is argued that this correlated response does not limit evolution within thermal environments, but instead may be one of the drivers of evolution and consequently biodiversity. The overall conclusion is that the biophysical Sharpe – Schoolfield equation is an excellent model to study thermal adaptation in ectotherms.

Keywords: *Thermal reaction norm, phenotypic plasticity, enzyme kinetics, temperature, development rate, growth rate, body size, Drosophila, anura, thermal adaptation, thermal tolerance limits, reversible temperature inactivation, cell cycle, Sharpe – Schoolfield equation, degree-day summation, tradeoffs*

Contents

CHAPTER 1

GENERAL INTRODUCTION

T.M. van der Have

Origin of life

Life probably originated at temperatures much higher than the temperatures, which sustain virtually all extant life forms (Di Giulio 2000 and references therein). This would suggest that from the very first beginning life evolved to be able to live at lower temperatures and that thermal adaptation primarily proceeds along the temperature axis. The first and foremost question which comes to mind is how ectotherms can cope with the over 100°C temperature range within which life occurs. The answer is that they simply cannot. Most eukaryotic ectotherms live between 0 and 40 °C, and usually the range of viable development is much less, in the region of 20°C. But even within these smaller ranges the effect of temperature on biochemical reactions and biological rates is substantial (6-10% per degree Celsius, Johnstone & Bennett 1996) and the question of how ectotherms cope with variable environmental temperatures is still justified.

Thermodynamics of chemical reactions

The observation of a high temperature dependence of biological rates follows from the empirical relationship between temperature and rates of chemical reactions described by the Arrhenius equation. The Arrhenius equation details an exponential increase of reaction rate with temperature. Eyring (1935) provided a theoretical foundation for this exponential relationship based on reaction kinetics. So, again, one could ask, how do ectotherms cope with this exponential increase in biological rates? Are they simply slaves to the tyrannical Eyring equation with limited possibilities for thermal adaptation to counteract or compensate for the exponential relationship? Or are they servants to the Eyring equation with a wide range of opportunities for thermal adaptation? The answer depends much on the function and flexibility of enzymes.

Enzyme activity and thermal performance

Enzymes reduce the activation energy required for reaction, while temperature influences the fraction of molecules with enough energy to react (Hochachka & Somero 1984, Hochachka 1991). A primary determinant of the inherent temperature sensitivity of any reaction is the enzyme catalytic efficiency. Enzymes which are highly efficient catalysts typically have low temperature sensitivity. There are so many factors, which can change the functioning of enzymes and thereby the temperature sensitivity of biochemical reactions that this could be considered as one of the megaproblems of ectothermy (Hochachka 1991). Any change in temperature may well differentially perturb a wide range of biochemical processes and integrating these effects to achieve an overall function is a huge problem for ectotherms.

When life evolved to ever lower temperatures enzymes had to become more and more efficient to compensate for the lower activity imposed by the tyranny of the Eyring equation. The enzymes of organisms living in warmer habitats (thermophiles) are generally less efficient than their homologous counterparts in colder habitats (Hochachka & Somero 1984). Enzyme function depends on a careful balance between structural stability, which determines

thermal range, and flexibility, which determines activity (Jaenike 1991, Somero 1995). Temperature affects both of these attributes and so proteins adapted to work at one temperature are inherently unable to maintain function at temperatures far removed from this optimum (Jaenike 1991, Fields 2001). This tradeoff in enzyme properties has pervading effects on the performance of ectotherms in a thermal gradient and suggests that the maximal enzyme activity of a eurytherm (thermal generalist) is always lower than that of a stenotherm (thermal specialist, Huey & Kingsolver 1993, Angilletta *et al.* 2003). Another important aspect of the thermal performance of ectotherms is the thermal limits of viable development. In a seasonal environment, for example, the lower thermal limit, often referred to as the threshold temperature for development, will determine the phenology or timing of appearance of a species. There is evidence that the temporal structuring of an aphidophagous (aphid eating) guild is caused by the differential effect of temperature on development rate (Dixon *et al.* 2005). Syrphid flies have a lower threshold temperature and always appear in summer before the coccinellid beetle larvae to exploit the peak in aphid abundance.

Climate change, species interactions and ecosystems

Temperature, through its thermodynamic effect on biochemical reactions, is a major factor governing the performance of ectothermic organisms in ecosystems worldwide. How can we start understanding and eventually predict temperature-induced changes of between-species interactions such as competition, symbiosis, predation, host-parasite interaction and plant-herbivore relationships? This question has become a focus of attention in ecology because global and regional climates continue to change: to become warmer and more variable. In the last decade, numerous studies have shown a wide range of effects of global and local warming on ecosystems (Petchey *et al.* 1999) and species interactions leading to a loss of production, and regional (Thomas *et al.* 2004) or global extinction of species (Pounds *et al.* 2006, Hughes 2000, Forchhammer & Post 2000).

The ecological impacts of recent climate change have been documented from polar terrestrial to tropical marine environments. These responses include both flora and fauna and span an array of ecosystems and organizational hierarchies, from the species to the community levels (Walther *et al.* 2002). Satellite data, for example, have shown that phytoplankton biomass and growth generally decline as the oceans' surface water temperature increases (Behrenfeld *et al.* 2006). Climate change can uncouple trophic interactions in aquatic ecosystems (Winder & Schindler 2004), shifts in the distribution of marine fishes (Perry *et al.* 2005), shifts in marine pelagic phenology and trophic mismatch (Edwards & Richardson 2004). Regional climate change has been reported to lead to mistiming between nesting date of a long-distance migratory bird and the spring food peak, leading to local population declines (Both *et al.* 2006, Visser *et al.* 1998). Pounds *et al.* (2006) have shown that widespread amphibian extinctions from epidemic disease are linked with global warming. These authors suggest that the temperatures at many highland localities are shifting towards the growth optimum of a fungus affecting the amphibians, thus encouraging outbreaks. Climate change has been implicated in shifts in species range (Davis *et al.* 1998), changed trophic interactions, Harrington *et al.* 1999, increasing incidence of pest outbreaks (Logan *et al.* 2003), changing host-parasite interactions (Bezemer *et al.* 1998), an increase in the incidence of coral bleaching (Kushmaro *et al.* 1996), an increase in the frequency of emerging marine diseases (Harvell *et al.* 1999) and emerging infectious diseases affecting human health (Epstein 1999). These are just a few examples of rapidly growing body of evidence for the effects of local and global temperature change on species interactions.

Life-history characters, reaction norms and tradeoffs

Species interactions can change if interacting species are differentially affected by temperature change in growth rate, development or differentiation rate, size at maturity and thermal limits. These life-history characters are (among others) important for reproduction, survival, rate of population increase and fitness within the thermal window of viable development (Huey & Berrigan 2001). The continuous function relating temperature to the phenotype expressed by a genotype is known as a reaction norm, which has become a unifying concept in evolutionary biology (Stearns 1989). The discovery of heritable variation in reaction norms within and among populations led to a range of theories designed to understand their evolution (Gotthard & Nylin 1995, Via *et al.* 1995, Schlichting & Pigliucci 1998). Adaptive explanations have been proposed, modelled and tested for much of the variation in life-history characters within and among species (Roff 2002). Genotypic models of reaction norms are particularly suitable to separate genotypic from environmental effects (Gavrilets & Scheiner 1993a, b, de Jong, 1990, 1995). But there is still the question which constraints limit the response to selection (Scharloo 1987) and which tradeoffs are involved. A tradeoff is a linkage between two traits that affects the relative fitness of genotypes and thereby prevents the traits from evolving independently (Angilletta *et al.* 2003). Three distinct types of tradeoffs can operate within the lifetime of an individual: (1) tradeoffs resulting from the allocation of available resources; (2) tradeoffs between minimizing mortality risk and maximizing resource acquisition and (3) tradeoffs resulting from environmental specialization (specialist – generalist tradeoff, Angilletta *et al.* 2003).

Several proximate mechanisms can result in tradeoffs, which can lead to genotypic differences between thermal reaction norms. A greater performance over a broad range of temperatures can be achieved by higher concentration of all isozymes (allocation or acquisition tradeoff). A greater performance over a narrow range of temperatures can be achieved by higher enzyme flexibility (specialist – generalist tradeoff). A greater performance at high temperatures can be realized with a higher stability of enzymes (generalist – specialist tradeoff). Angilletta *et al.* (2003) argue that a unified theory that includes all classes of tradeoffs would provide a better understanding of the mechanisms that drive the evolution of reaction norms. Considering the importance of enzyme properties for most classes of tradeoffs, it seems clear that a biophysical model, which describes the temperature dependence of biological rates based on the thermodynamics of reaction kinetics, should be an essential part of that unifying theory.

A biophysical model for temperature dependence

The first step would be to model the general temperature dependence of biological rates. The second step is to find explanatory and mechanistic models to predict temperature dependence of species-specific characters such as growth rate, development rate, body size and thermal limits. Finally, these models could be used to explore and explain patterns in thermal adaptation within and among species.

Sharpe & DeMichele (1979) applied Eyring's theory to a unified rate model that describes the rate of biological rate processes for all temperatures that support life. Most biological rates do not increase exponentially with temperatures as in chemical reactions, but increase quasi-linear above a certain threshold up to a maximum rate (Figure 1). Sharpe & DeMichele (1979) proposed that reversible inactivation at high and low temperatures linearize the exponential Eyring equation over much of the thermal range and, therefore, provide a

mechanistic model for biological rates within species. Their model is particular suited to describe poikilotherm development in particular, such as differentiation rate, cell division rate or growth rate. Their model is derived from Johnson and Lewin (1946), and in its basic form already proposed by Briggs and Haldane (1925). The model is based on the thermodynamic properties of a system acting as a single, hypothetical, developmental enzyme that is rate limiting to development. This rate-controlling enzyme is assumed to be characterized by a constant molecular population which exists either in active form (at normal temperatures) or in reversibly inactive forms (at high or low temperatures). The biophysical model differs in this respect from thermal performance', where only the decrease in reaction rates at higher temperatures is linked with thermal instabilities of enzymes (Hochachka and Somero, 1984; Heinrich, 1977). The Sharpe – Schoolfield model can describe the temperature dependence of biological rates within species and can provides us with insights in the proximate mechanisms of thermal adaptation. The thermodynamic parameters can also easily be linked to heritable variation (De Jong & Imasheva 2001).

Comparisons between species would involve rate comparisons at the midpoints of the temperature ranges of the species, and presumably not involve enzyme inactivation. The central role of the Eyring equation in the Sharpe – Schoolfield equation makes it therefore a good choice for cross-species comparisons, better than the Sharpe – Schoolfield model itself. The Eyring equation is in its basic form very similar to the equation for universal temperature dependence (UTD) proposed by Gillooly *et al.* (2001), but its central role in the Sharpe – Schoolfield equation makes it a better choice for cross-species comparisons, as then the link with within species processes is obvious.

A life-history puzzle: temperature effects on growth rate and body size

Experiments have shown that a lower environmental temperature causes an increase in adult size in over 80% of the species studied to date (Atkinson 1994, 1995, Atkinson *et al.* 2003). This thermal plasticity of size at maturity has been observed in bacteria, protests, plants and animals, also known as the temperature – size – rule, and is probably one of the most taxonomically widespread rules in biology (Angilletta *et al.* 2004). This rule also seems to apply to egg size: ectotherms, including crustaceans, insects, fish, amphibians and retiles, often produce larger eggs at lower temperature (Blanckenhorn 2000, Fischer, Brakefield & Zwaan 2003, Yampolski & Scheiner 1996). The relationship between temperature and life history characters have puzzled evolutionary ecologists because of the paradoxical effects of temperature on growth rate and size: lower temperatures cause ectotherms to grow slower but mature at a larger size. On the other hand, classic theories of life-history evolution predict a smaller size at maturity in environments that cause growth to proceed slower (reviewed by Berrigan & Charnov 1994). Many theoretical and empirical studies have been carried out in the last decade and have generated several plausible proximate or ultimate explanations, but apparently no single theory has been able to explain widespread occurrence of the temperature – size – rule in ectotherms. This prompted Angilletta *et al.* 2004 to recommend a multivariate theory that incorporates both functional constraints on thermal reaction norms and the natural covariation between temperature and other environmental factors. Again, the biophysical Sharpe – Schoolfield model seems a logical choice to model the functional constraints on reaction norms imposed by temperature.

Outline of the thesis

The general question of my thesis is to what extent the thermodynamics of biological rates constrain the thermal adaptation of developing ectotherms. I approach this problem at two levels, the patterns in thermal adaptation within and among species, and focused on four research questions:

1. Why do most ectotherms become smaller when growing faster at higher temperatures and larger when growing slower at lower temperatures? Is there a general model to explain the Temperature Size Rule (TSR) in ectotherms?
2. Why are temperature limits in developing ectotherms usually steep and well-defined at both low and high temperatures? Is there a single model which applies to both thermal limits?
3. How can one predict patterns in thermal adaptation within and among species from the kinetics of reaction rates?
4. Are linear temperature-development rate reaction norms simply approximations of the general temperature dependence predicted by the Eyring equation?

The first two questions refer to phenomena occurring in most, if not all ectotherms, the last two questions deal in particularly with patterns among species. I suggest that these questions can be tackled by the application of the biophysical Sharpe - Schoolfield model to describe the temperature dependence of biological rates, in particular to reaction norms of life-history characters, such as embryonic and larval development rates, growth rate and size at maturity.

It should be noted that the use of the words development rate and differentiation rate differs slightly among the chapters. In Chapter 2 and 3 it is argued that development can be thought of as consisting of two different components, differentiation and growth. At the cellular level these two processes are represented by cell division and cell growth, respectively, and their interaction will eventually determine the size at maturity (Bonner 1952, Clarke 1967, Needham 1964, Ratte1984, Wigglesworth 1953). Both chapters describe models that make explicit assumptions about these two processes, which justify the use of differentiation rate instead of development rate. Chapter 4 and 5 are mainly concerned with among species comparisons and make many comparisons with other ecological and evolutionary studies which almost invariably use development time or rate and often without any reference to the fact that growth and differentiation are two very different components of development.

I propose in Chapter 2 a proximate, biophysical model that predicts the temperature – dependent size variation of ectotherms at maturation from the difference in temperature dependence of growth and development. The Sharpe – Schoolfield model is used to describe the temperature – modulated variation in growth and development rate and which are both integrated in a simple growth model. This biophysical model can provide a proximate framework for genotypic models of reaction norm evolution. Genetic variation in either growth or development rate reaction norm would lead to genotype by environment interaction.

In Chapter 3 I propose a proximate model for thermal tolerance limits in developing ectotherms, which shows that the interaction between reversible temperature inactivation of cell cycle proteins and their regulation can explain the symmetry and threshold character of these thermal limits. The model suggests that temperature inactivation of regulatory proteins mimics the decrease in concentration resulting from gene dosage change and transcriptional

regulation during the cell cycle. If the activity of certain regulatory proteins is halved by temperature inactivation then cell division and, consequently, development becomes blocked. The thermal limits expected from this model were compared with thermal limits in 23 insect species and were found to agree closely in 21 comparisons.

Chapter 4 compares the Sharpe – Schoolfield model with the thermal time concept and the transformation of physical time to physiological time, reviews how genetic variance in development rate, growth rate and body size over temperature results from genetic variation in the biophysical parameters and explores the possibilities of the Sharpe – Schoolfield model to explain geographical clines in adult body size. It is demonstrated how variation in the model parameters can be used to model genetic variation within and between populations.

Chapter 5 continues to focus on the question to what extent thermodynamics constrains thermal adaptation in developing ectotherms, in particular, in comparisons among species. It is explored how the major patterns of thermal adaptation can be generated by varying the parameters of the Sharpe – Schoolfield equation. The special case of developmental rate isomorphy (Jarošik *et al.* 2002, 2004), is addressed. This refers to the observation that in many insect species temperature sensitivity varies among developmental stages while the threshold temperature remains constant. The model predictions are used to analyze patterns of thermal adaptation in approximately fifty species of anurans. This group of ectotherms provides an excellent data set, because such a complete set of information is available in the literature, including experimentally determined thermal limits of development and embryonic developmental rates. This data set is also used to test the proposition of Gillooly *et al.* (2001, 2002) that the temperature dependence of metabolic and developmental rates comply to a Universal Temperature Dependence. UDT is based on the empirical Arrhenius equation and literature data on the activation energy of biochemical reactions.

Chapter 6 takes another look at the underlying cellular processes of growth and development, which are fundamental to the assumptions of the proximate models for the temperature dependence of size at maturity and thermal limits proposed in chapter 2 and 3. The recent developments in evolutionary ecology with respect to understanding the underlying mechanisms and adaptive significance of the Temperature Size Rule, the genetics of plasticity and the physiological processes determining thermal limits in ectotherms are reviewed. In addition, I explore the consequences of the findings in chapters 2-5 for our understanding of the effect of environmental temperatures and temperature change for species interactions and host – parasite relationships in particular. Finally, I discuss the implications of thermodynamic constraints in thermal adaptation of developing ectotherms and of the importance of reaction norms in evolution.

References

See Chapter 6.

CHAPTER 2

ADULT SIZE IN ECTOTHERMS: TEMPERATURE EFFECTS ON GROWTH AND DIFFERENTIATION

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ABSTRACT

A proximate, biophysical model is proposed describing temperature-modulated variation in growth rate and differentiation rate in ectotherms, based upon the Sharpe-Schoolfield equation connecting enzyme kinetics and biological rates. Like the Sharpe-Schoolfield equation, the model assumes 1) that growth rate and differentiation rate can be described as controlled by one rate-limiting enzyme; in addition, the model assumes 2) that the temperature coefficients of growth and differentiation are different. The model is used to predict temperature-dependent size variation at maturation of ectotherms as a result of the interaction of growth and differentiation. It is shown that the difference between the activation energy constants of growth and differentiation determines the slope of the size-temperature reaction norm within the range of normal development. The structural and heritable variation in enzymes determines reaction norm shape without inferring regulatory genes. All thermodynamic parameters of the Sharpe-Schoolfield equation can be estimated empirically with non-linear regression techniques. The biophysical model provides a proximate framework for genotypic models of reaction norm evolution; genetic variation in either growth or differentiation would lead to genotype by environment interaction. This proximate model of temperature sensitivity and temperature tolerance clarifies how temperature dependence of body size would evolve.

Key words: *Reaction norm, enzyme kinetics, temperature, growth rate, differentiation rate, Drosophila.*

INTRODUCTION

Size at maturity and growth rate are key traits in life-history evolution (Roff, 1992; Stearns, 1992; Charnov, 1993). Both size at maturity and growth rate are subject to environmental variation. Environmental variation can be included in the models of life-history evolution by way of a reaction norm, a continuous genotypic function which maps the environment onto phenotype. Here we will be concerned with what determines the shape of the reaction norm of growth rate, development rate and size at maturity as a function of temperature, focusing upon the often observed decrease in size with higher developmental temperatures.

The environment considered in most life-history models is variation in resource level. Temperature effects on size at maturity in ectotherms may be very different from the effects of resource variation. For example, the size range in *Drosophila melanogaster* is smaller when developmental temperatures vary (David & Clavel, 1967), relative to size variation induced by different food levels during larval growth (Bakker, 1961): the thermal "window" of viable development is narrower than the resource "window". The reverse has been found in amphibians (Smith-Gill & Berven, 1979). In several groups of ectotherms temperature has been indicated as the major proximal factor explaining the variation in growth rate and development rate (*e.g.*, copepods, Huntley & Lopez, 1992; amphibians, Smith-Gill & Berven, 1979). Whether these differences in the effect of environmental factors during development are adaptive or constraints (*sensu* Oster *et al.*, 1988) is not clear, but the distinction is evidently essential when size variation in a variable environment is interpreted in an evolutionary context (Roff, 1981; Newman, 1992).

Recent empirical work (*e.g.*, Reznick, 1990) has revealed much complexity in the environmental effects on growth and development not anticipated by theoretical life-history models (Roff, 1992; Stearns, 1992), and calls for more detailed genetic analyses and experimental studies designed to test the developmental basis of maturation and its sensitivity to environmental factors (Bernardo, 1993). For example, size-dependent survival and fecundity are focal parameters in ultimate, life-history, models and are used to predict optimal size (Roff, 1981). This approach assumes that body size is not constrained by proximate environmental factors. However, in many if not most ectotherms size at maturity has been found to decrease with increasing growth temperature when food is applied *ad libitum* (reviewed by Atkinson, 1994), pointing to the importance of the differential effects of temperature on the interaction between growth and differentiation as has been first suggested by Smith-Gill and Berven (1979).

This paper presents a proximate, biophysical model to describe the shape of reaction norms for temperature and physiological rates such as growth and differentiation and to predict the temperature-dependence of size at metamorphosis. The main focus will be on organisms with determinate growth, in particular holometabolic insects like *Drosophila*, but the model may also apply to the temperature-dependence of size at maturity for ectotherms with indeterminate growth. In addition, the implications for quantitative genetic models for the evolution of reaction norms will be discussed.

BIOPHYSICAL MODEL FOR SIZE AT METAMORPHOSIS

Growth and differentiation

During the development from zygote to metamorphosis the tissue of an organism differentiates and expands through a sequence of cell divisions and cell growth.

Consequently, development can be thought of as consisting of two different components, differentiation and growth (Bonner, 1952; Clarke, 1967; Needham, 1964; Ratte, 1984; Wigglesworth, 1953). The two processes together will eventually determine the size of the organism at metamorphosis (Berven *et al.*, 1979; Smith-Gill & Berven, 1979). It will be argued that these developmental components are not simply two sides of the same coin, but are driven and controlled by different and functionally separate mechanisms.

Growth is increase in biomass and growth rate has the dimension of biomass *m* per unit time *t* irrespective of developmental stage. Differentiation is the diversification of cell types during development and proceeds primarily by cell divisions. Differentiation rate is expressed as the reciprocal of the time between hatching and metamorphosis ($time^{-1}$). Cells will usually stop dividing when they are terminally differentiated. The differentiation rate can be defined as the number of developmental stages an organism "goes through" per unit time. In organisms with an invariant number of cells in the adult stage (*e.g.*, Rotifera) every developmental stage could be defined by the number of cells. Adult body size variation would only result from cell size variation. In organisms varying in adult size through variation in cell number and cell size each subsequent developmental stage could be defined by either morphological characters, or by a combination of cell number and cell types. Note that cell number and any other representations of developmental stages are dimensionless variables.

In this paper we consider protein synthesis as the major aspect of growth and DNA replication as the major component of the cell division cycle relevant to the temperature dependence of development. The start of the cell cycle, leading to DNA replication and eventually mitosis, is regulated by cell cycle proteins Cdc2 and cyclin (forming a heterodimer called the M-phase promoting factor MPF) and enzymes like Wee1 and Cdc25 controlling the phosphorylation state of Cdc2 (Tyson, 1991; Novak & Tyson, 1993; Murray, 1992; Hartwell & Weinert, 1989). There is generally no fixed cell size that triggers the starts of events leading to mitosis, only a minimum viable cell size (Novak & Tyson, 1993). This suggests that at the cellular level growth and cell division are only loosely connected (Sennerstam & Strömberg, 1995). For example, during embryonic development in many ectotherms only cell division and differentiation occurs without growth. During subsequent larval stages differentiation can be easily uncoupled from growth in most arthropods by hormone treatment, for example by inducing moult. And, evidently, the timing of maturation or metamorphosis in most ectotherms is also closely under hormonal control. There are some suggestions, however, that the timing of the underlying process primarily depends on the sequence of cell divisions (Satoh, 1982; Holliday, 1991).

If cells divide faster and the organism differentiates more rapidly, while cellular growth rate remains constant, the resulting adult will be smaller due to a smaller average cell size. Variation in cell size of full grown organisms has been well studied in *Drosophila* and found to be influenced by environmental conditions during development in particular temperature (Alpatov, 1930; Delcour & Lints, 1966; Masry & Robertson, 1979; Partridge *et al.*, 1995; Robertson, 1959). In most of these studies a higher growth temperature resulted in smaller sized adults mainly because of a smaller average cell size.

The key issue in this paper is how size at metamorphosis is determined by temperature during development. It will be argued that because of intrinsic differences between differentiation (DNA replication) and growth (protein synthesis) the temperature coefficients can expected to be different. A simple model is developed to show how temperature-dependent size variation can be explained by the thermodynamic properties of differentiation and growth.

If growth is represented in its simplest form as a linear increase in biomass per unit time (as in Smith-Gill & Berven, 1979; Alford & Jackson, 1993), then size or biomass at metamorphosis *m* can be expressed as:

$$m = m_0 + Gt \quad (1)$$

where m_0 is the biomass at hatching, G is growth rate (mass increase per unit time) and t is the time between hatching and maturation or metamorphosis. For the reasons explained above it is convenient to express differentiation as a rate, D, which is the reciprocal of time between hatching and metamorphosis ($1/t$ or unit time^{-1}). In that case mass at metamorphosis m will be:

$$m = m_0 + \frac{G}{D} \quad (2)$$

SHARPE-SCHOOLFIELD EQUATION

Both growth rate and development rate depend on environmental factors of which only temperature will be considered. To develop a model for the temperature dependence of size at metamorphosis, m, we assume that differentiation and growth rate are independent at all temperatures under non-limiting food conditions. Under this assumption growth rate and differentiation rate can be expressed as temperature-dependent processes in terms of enzyme kinetics, but with different thermodynamic constants. Sharpe & DeMichele (1977) derived a biophysical model to describe the temperature-dependence of any aspect of poikilotherm development, such as differentiation rate, cell division rate or growth rate. Their model is derived from Johnson & Lewin (1946), and in its basic form already proposed by Briggs & Haldane (1925). The model is based on the thermodynamic properties of a single, hypothetical, developmental enzyme which is rate-limiting to development. This developmental enzyme is assumed to be characterized by a constant molecular population which exists either in active form (at normal temperatures) or in reversibly inactive forms (at high or low temperatures). By combining the Eyring equation with reaction rate kinetics Sharpe & DeMichele (1977) and Schoolfield *et al.* (1981) derived an equation (the Sharpe-Schoolfield equation) for any rate of development under non-limiting substrate conditions:

$$r(T) = \frac{\rho T P_T}{298.2} \exp\left(\frac{\Delta H^{\neq}_A}{R}\left(\frac{1}{298.2} - \frac{1}{T}\right)\right) \quad (3)$$

where $r(T)$ is the mean development rate (days^{-1}) at temperature T (K), P is the probability that the rate controlling enzyme (of growth or differentiation) is in active state, R is the universal gas constant (1.987 cal deg^{-1} mol^{-1}) and 298.2 is a standard reference temperature in degrees Kelvin (equivalent to 25°C). The thermodynamic parameters are as follows: ρ is the development rate (days^{-1}) at the standard reference temperature of 25°C assuming no enzyme inactivation (this implies that 25°C is the optimal temperature), $\Delta H^{\neq}_A$ is the enthalpy of activation (cal mol^{-1}) of the rate controlling enzyme. The probability P that the rate controlling enzyme is in active state is defined by:

$$1/P_T = 1 + \exp\left(\frac{\Delta H^{\neq}_L}{R}\left(\frac{1}{T_{\frac{1}{2}_L}} - \frac{1}{T}\right)\right) + \exp\left(\frac{\Delta H^{\neq}_H}{R}\left(\frac{1}{T_{\frac{1}{2}_H}} - \frac{1}{T}\right)\right). \quad (4)$$

where $T_{½H}$ and $T_{½L}$ are the temperatures (°K) at which the enzyme is half active and half inactive by high or low temperatures, respectively, $\Delta H^{\neq}_H$ and $\Delta H^{\neq}_L$ are the change in enthalpy (cal mol^{-1}) associated with respectively high or low temperature inactivation of the enzyme.

The Sharpe-Schoolfield equation has considerable advantage over other expressions of biological rate functions (reviewed in Wagner *et al.* (1984). It can accurately describe the temperature dependence of a developmental process over the whole range of biological activity, including the quasi-linear region at intermediate temperatures and the non-linear regions at high and low temperatures (Fig. 1). Reversible inactivation of enzymes approximately linearizes the exponential rate function expected from the Eyring equation. It should be noted that the maximum rate occurs well above the optimum temperature of 25°C, which is defined as the temperature at which no inactivation of the hypothetical developmental enzyme occurs. However, the rate function apparently does not relate to the discrete thermal limits of development in ectotherms (Fig. 1).

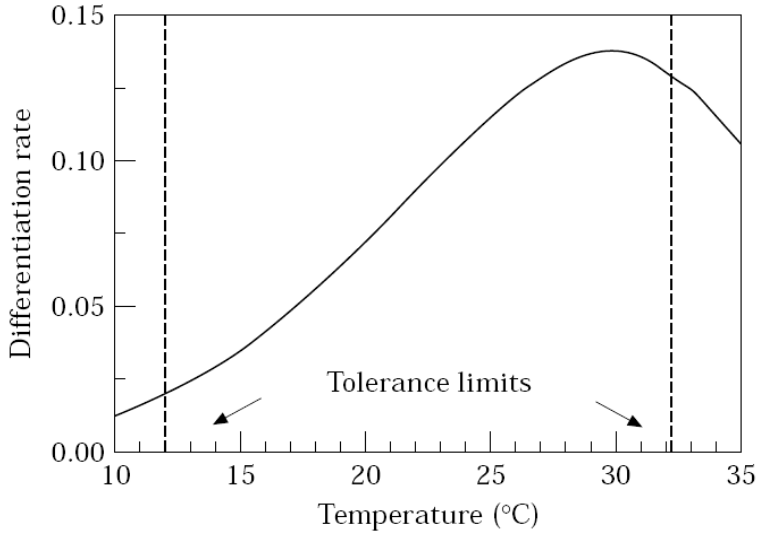

Fig. 1. General shape of the Sharpe-Schoolfield equation describing the temperature dependence of a developmental rate (time^{-1}), such as growth rate, differentiation rate, or cell division rate. Note the semi-linear region around standard reference temperature of 25°C and the thermal tolerance limits for larval development (broken lines).

The thermal range of normal development in most ectotherms is from about 10°C to about 30°C. Genetic variation in the enthalpy of activation, $\Delta H^{\neq}_A$, leads to genetic variation in rate, and to genetic variation in the rate functions $r(T)$ (Fig. 2). The rate functions can be considered as the reaction norms of developmental rate or growth rate; they are non-parallel and cross at the standard reference temperature of 25°C. Genetic variation in the enthalpy of activation leads to genotype by environment interaction in biological rates.

If both growth rate G (mass per unit time) and differentiation rate D (developmental stages per unit time) are expressed as functions of temperature according to equation (3) and substituted in equation (2), mass at metamorphosis as a function of temperature T will be:

$$m_t(T) = m_0 + \frac{\rho_g P_g}{\rho_d P_d} \exp\left(\frac{\Delta H^{\neq}_{A,g} - \Delta H^{\neq}_{A,d}}{R}\left(\frac{1}{298.2} - \frac{1}{T}\right)\right), \qquad (5)$$

where the subscripts refer to growth rate (*g*) or differentiation rate (*d*). ρ_g is growth rate (mass·time^{-1}) and ρ_d is differentiation rate (time^{-1}) at the standard reference temperature of 25°C. As mentioned before, differentiation and growth are assumed to have different rate-limiting enzyme reaction steps.

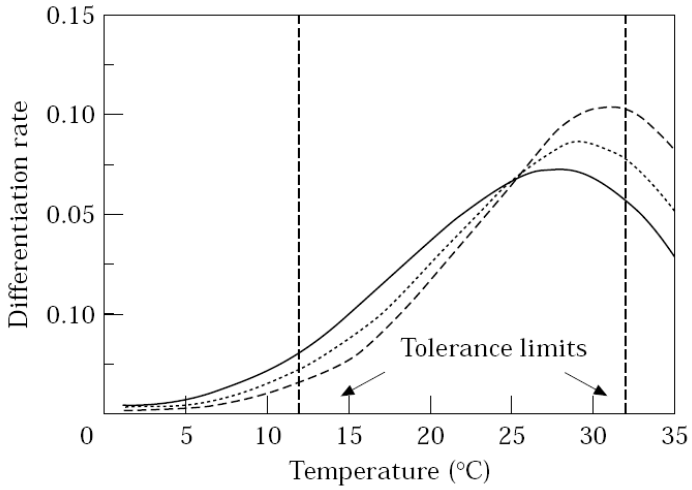

Fig. 2. Variation in shape of the reaction norm resulting from the Sharpe-Schoolfield equation by variation in enthalpy of activation ($\Delta H^{\neq}_A$) of the developmental enzyme and keeping the other thermodynamic parameters constant. Note that the reaction norms cross at 25°C. This would imply clear genotype by environment interaction if the variation in $\Delta H^{\neq}_A$ is genetically determined.

Smaller size at metamorphosis or maturation in ectotherms grown at higher temperatures but under surplus food conditions is a widely observed phenomenon (Bělehrádek, 1935; von Bertalanffy, 1960; Ray, 1960; Precht *et al.*, 1973; Atkinson, 1994*a*; marine copepods, Huntley & Lopez, 1992; Moore & Folt, 1993 and references therein; butterflies, Oldiges, 1959; *Drosophila*, David & Clavel, 1967; agronomic yield in crops Atkinson, 1994b). This "biological law" can be phrased as a question: under what conditions will the slope of $m(T)$ become negative? In other words, at which parameter combinations would $\partial m(T)/\partial T$ be less than zero, at least within the thermal tolerance limits of development?

An answer to this question can be based upon equation (5). It can be assumed that high and low temperature inactivation approaches zero respectively below and above 25°C. In addition, assume for the sake of simplicity that $T_{½,L,d} = T_{½,L,g}$ and $T_{½,H,d} = T_{½,H,g}$. Then, size at metamorphosis will be a decreasing function of temperature if the following inequalities are satisfied:

$$\Delta H^{\neq}_{A,d} - \Delta H^{\neq}_{A,g} > (1 - P_d)\Delta H^{\neq}_{H,d} - (1 - P_g)\Delta H^{\neq}_{H,g} \qquad (for\ T > 298.2°K) \qquad (6a)$$

$$\Delta H^{\neq}_{A,d} - \Delta H^{\neq}_{A,g} > (1 - P_d)\Delta H^{\neq}_{L,d} - (1 - P_g)\Delta H^{\neq}_{L,g} \qquad (for\ T < 298.2°K) \qquad (6b)$$

In words, the slope of the reaction norm is negative if the difference in temperature sensitivity

of the ongoing processes is higher than the difference in temperature sensitivity of the inactivation of the processes. Since the probability P_T that the rate controlling enzyme is in active state is itself temperature dependent, size at metamorphosis might increase for some temperature range and decrease for another temperature range. More generally, conditions 6(a)-(b) suggest that the difference between the activation energy constants of growth and differentiation determines the slope of the size-temperature reaction norm within the temperature range of normal development. The temperature coefficient of growth should be lower than the temperature coefficient of differentiation for the size of ectotherm animals to decrease with increasing temperature.

DISCUSSION OF ASSUMPTIONS

The described biophysical model predicts size at metamorphosis within the "thermal window" of development, that is within the tolerance limits of development. It consists of two similar expressions for temperature dependence of differentiation rate and growth rate, which respectively determine timing and scale of development. To evaluate how much biological realism the model contains, a closer look is necessary, first at the four assumptions, second to see if the predictions are testable.

The first assumption, that one reaction step is rate limiting, is already present in the Sharpe-Schoolfield equation (3). Although the validity of the concept that one reaction step is rate-limiting to either growth or differentiation has been questioned (*e.g.*, Ratte, 1984; Lamb, 1992), no theoretically based alternatives have been put forward. Sharpe & DeMichele (1977) have graphically shown that the shape of the temperature-dependent rate function is relatively insensitive to the occurrence of more than one rate-limiting enzyme reaction. In the absence of more detailed information it seems best to assume that the system from input to output might function as if one enzyme reaction is rate-limiting. Therefore, the thermodynamic parameters should be seen more as characteristics of the system than of a particular enzyme. This is supported by the observation of Craig & Fahrman (1977), who found that the temperature dependence of protein synthesis was not rate-limited by some membrane phenomenon, but due to some factor inherent to the process such as reversible inactivation.

The second assumption is that growth rate and differentiation rate have different temperature coefficients. Hochachka and Somero (1984) list known thermodynamic parameters: it is a range of values, and variation clearly exists in the thermodynamic properties of enzymes. Certainly, if growth and differentiation had totally the same thermodynamic parameters, it would be an evolutionary question why that should be the case. In equation (5), identical thermodynamic parameters would indicate that biomass would not change from m_0. However, the conditions (6) indicate that the real biological question is why the temperature coefficient $\Delta H^{\neq}_{A,g}$ of growth should be lower than the temperature coefficient $\Delta H^{\neq}_{A,d}$ of differentiation? A possible argument centers on the temperature coefficients of protein synthesis and DNA replication: we assume that growth rate depends primarily upon the rate of protein synthesis, and differentiation rate upon the rate of DNA replication. Protein synthesis differs from DNA replication with respect to the size of the molecules involved. The ribosomal subunits are huge molecules (molecular weights of 40S and 60S subunits 1,500,000 and 3,000,000, respectively) in comparison with the much smaller DNA polymerases (molecular weights between 110,000 and 180,000). This implies that the large ribosomal subunits diffuse slower into the cytoplasm, before asssembly into ribosomes, than the DNA polymerases within the nucleus. They need more time to form complexes with mRNA and other subunits than the DNA polymerases do to find the DNA template (Xia, 1995). Diffusion

processes are generally independent of temperature (Q_{10} close to 1, Hochachka, 1991). DNA replication will depend upon the enzymatic speed of the polymerases, with a Q_{10} nearer to 2. Therefore, as diffusion is more rate-limiting in protein synthesis than in DNA replication the temperature coefficient of growth can be expected to be lower than the temperature coefficient of differentiation. There is some supportive empirical evidence for this in the carabid beetle *Notiophilus biguttatus* (Ernsting & Huyer, 1984). Egg development (mainly differentation) was found to be more sensitive to temperature than larval growth (mainly protein synthesis).

A third assumption of the biophysical model is that the focal enzyme in equation (3) occurs reversibly in active or inactive state. The optimal temperature at which little or no inactivation is occurs, is arbitrarily chosen as 25°C (298.2°K, Schoolfield *et al.*, 1981), although any temperature between 20° and 30°C would be appropriate for most organisms. A particular realistic property of equation (3) is that the maximum growth or differentiation rate always occurs well above the temperature of maximal enzyme performance (25 °C in the example above) and usually near the upper thermal limit of development. This is intuitively appealing, because optimal temperatures of many life-history characters are usually intermediate between the thermal limits of development and below the temperature of maximum performance (Huey *et al.*, 1991; *e.g.*, ovariole number and egg production in *Drosophila*, David *et al.*, 1983).

In the model the simplest possible type of growth is chosen as the fourth assumption: a linear increase of biomass in time. An exponential model of growth (*e.g.*, Bakker; 1961, Huntley & Lopez, 1992), with size at metamorphosis as $m=m_o e^{\gamma/D}$, where γ is the instantaneous growth rate with temperature dependence following equation (3), leads to essentially the same conditions [equations 6(a,b)] for the slope of the size-temperature reaction norm to be negative. It should be noted in this context that non-linear models (logistic, Gompertz or von Bertalanffy, Reiss, 1989) are widely applied to describe ectotherm growth. However, growth rate usually declines when reproduction starts (Roff, 1980; Charnov, 1993 p.141-142) and up to this moment, that is maturation or metamorphosis, non-linear models are indistinguishable in performance from exponential or even linear models to describe growth (Reiss, 1989).

AN EXAMPLE: *DROSOPHILA MELANOGASTER*

The behaviour and predictions of the model can best be illustrated by an example in which the thermodynamic constants of both growth and differentiation are estimated. Coefficients estimated on the basis of the Sharp-Schoolfield equation (3) are substituted in equation (5) for comparison with experimental data on size at metamorphosis. Two studies on *Drosophila melanogaster* are suitable as they have reported the duration of development and weight at eclosion over the total thermal range (Cohet *et al.*, 1980; David & Clavel, 1967; respectively).

To illustrate the usefulness of equations (3) and (5) we calculated average growth rate as the average weight at eclosion (data from David & Clavel, 1967) divided by average duration of development (data from Cohet *et al.*, 1980; c.f. Emerson *et al.*, 1988; Hillesheim & Stearns, 1991), and average differentiation rate as the inverse of the average duration of development. The resulting functions describing the temperature dependence of growth rate and differentiation rate (fig. 3) resemble qualitatively the pattern found in other organisms with metamorphosis such as in the frog *Rana clamitans* (Berven & Smith-Gill, 1979), and the blowfly *Lucilia illustris* (Hanski, 1976): an increase up to a relatively high temperature and a sharp decrease near the upper limit of larval development .

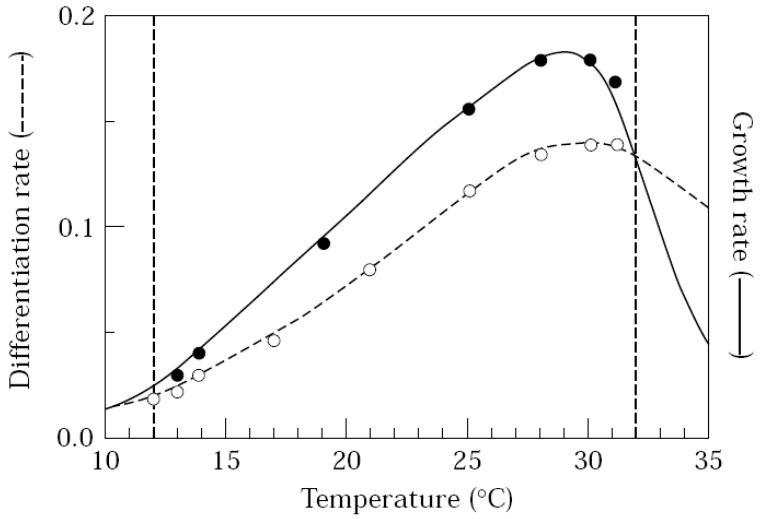

Fig. 3. Temperature dependence of differentiation rate and growth rate. Observed differentiation rates (open circles) and growth rates (solid circles) are compared with predicted rates (dotted line: differentiation rate, solid line: growth rate). The thermodynamic parameters of the Sharpe-Schoolfield equation were estimated with nonlinear regression and are presented in Table 1. The dotted lines refers to the observed thermal limits of larval development. Data from Cohet *et al.*, 1981 and David & Clavel, 1967).

Table 1. Parameter estimates of the biophysical model for differentiation rate and growth rate of *D. melanogaster*

	ρ	$\Delta H^{\neq}_A$	$T_{½L}$	$\Delta H^{\neq}_L$	$T_{½H}$	$\Delta H^{\neq}_H$
	$days^{-1}$	cal/mol	°K	cal/mol	°K	cal/mol
Differentiation rate	0.136	15,789	285.3	-42,232	305.3	52,730
Growth rate	0.16	9,465	287.3	-59,455	305.4	138,164

The thermodynamic constants of differentiation and growth rates were estimated with non-linear regression (SAS, 1988) applying the Sharpe-Schoolfield equation (Table 1); it was assumed that no high and low inactivation occurs at 25°C. This temperature can be considered as optimal for *D. melanogaster*, as, for example, maximal population growth occurs (Siddiqui & Barlow, 1972). All estimates fall within the range of thermodynamic parameters known for enzymes in a wide range of organisms (Hochachka & Somero, 1984). Fig. 4 shows the temperature dependence of weight (size) at eclosion if all estimated thermodynamic parameters are substituted into equation (5). The close fit with the observed data from David & Clavel (1967) is of course not very surprising considering the way in which growth rate was calculated, but clearly illustrates the properties of the model if realistic values for the thermodynamic constants are applied. This combination of parameters shows a maximum size at a low temperature (around 17°C), a decrease with increasing temperature and a sharp decrease near the upper and lower temperature limits of development (12°C and 31°C, respectively).

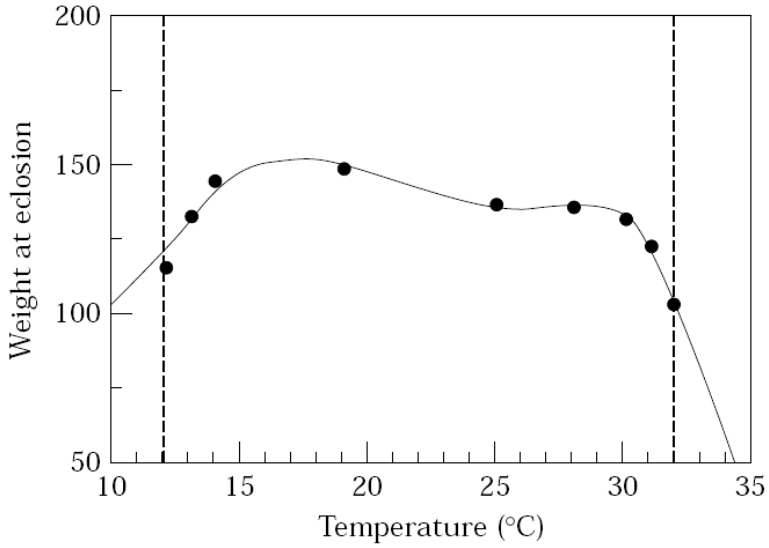

Fig. 4. Size at metamorphosis observed under controlled conditions as a function of temperature (dots: data from David & Clavel, 1967) compared with the prediction from equation (5) (line) to illustrate the biophysical model.

Discussion

FROM ENZYME KINETICS TO GENETIC VARIATION IN REACTION NORMS

In the discussion of a general quantitative genetic model for the evolution of reaction norms, Gavrilets & Scheiner (1993*a*) suggested that in order to develop realistic genotypic models for the evolution of reaction norms we need to know how the individual components of the developmental program translate into reaction norms at the whole organism level. More specifically, they posed the question how differences in the reaction rates of enzymes as a function of temperature combine to produce a reaction norm, if a trait is determined by a series of reaction steps. The model presented here can be seen as a first step towards an answer. Growth and differentiation are considered as two fundamental and parallel components of development determining size at maturation. Emphasis is laid on the interaction between the processes of growth and differentiation.

The temperature reaction norms of each of these components can be described by the Sharpe-Schoolfield equation (3) and its biophysical parameters. These parameters are interpretable in terms of biochemical adaptation (*e.g.*, Hochachka & Somero, 1984; Powers, 1993), although the extension from one rate-limiting reaction to a chain of reactions needs further study.

All thermodynamical parameters of the Sharpe-Schoolfield equation are rooted in enzyme kinetic theory (Schoolfield *et al.*, 1981; Wagner *et al.*, 1984). The thermodynamic constants both for activation and inactivation have been published for numerous enzymes (*e.g.*, Hochachka & Somero, 1984). The thermodynamic constants estimated with non-linear regression methods on the basis of equation (3) from published data of several insect species fall well within this range (Sharpe & DeMichele, 1977; Schoolfield *et al.*, 1981; and

references in Wagner *et al.*, 1984; van Straalen, 1994, 1995).

Variation in the thermodynamic parameters of the Sharpe-Schoolfield equation can originate from structural and heritable variation in the enzymes determining the temperature dependence of growth rate or differentiation rate. What are the effects of this variation on the shape of the reaction norm for temperature dependence in growth and differentiation rate? The parameters of interest appear in the conditions for the slope of the reaction norm of size at metamorphosis as a function of temperature to be negative [equations 6(a)-(b)]: these conditions define the central problem of this paper. Variation in the change of enthalpy by high and low temperature inactivation (respectively $\Delta H^{\neq}_H$ and $\Delta H^{\neq}_L$) mainly influences the shape of the non-linear region of the Sharpe-Schoolfield rate function. The subsequent effect on size at metamorphosis is mainly found near the thermal limits of development.

The Sharpe-Schoolfield equation can be trimmed down to a two-parameter model, by setting $P_g=P_d=1$: this further assumption identifies the enthalpy of activation as the crucial parameter in the near optimal temperature region with little temperature inactivation. Equation (5) then reduces to a four-parameter equation, and the slope with temperature of size at metamorphosis becomes negative if $\Delta H^{\neq}_{A,d} - \Delta H^{\neq}_{A,g}$ is larger than zero. This implies that the difference in temperature coefficients of growth rate and differentiation rate will largely determine the slope of size at metamorphosis with temperature, within the range of normal development. This property can be illustrated in the six-parameter model if either $\Delta H^{\neq}_{A,g}$ or $\Delta H^{\neq}_{A,d}$ is varied, keeping the other parameters constant. The resulting sizes at metamorphosis are shown in Fig. 5. The reaction norms vary as predicted and cross at the chosen optimal temperature of 25°C. This result shows that genotype by environment interaction in growth rate or differentiation rate or in both, due to genetic variation in thermal constants, can contribute directly to genotype environment interaction in size at metamorphosis.

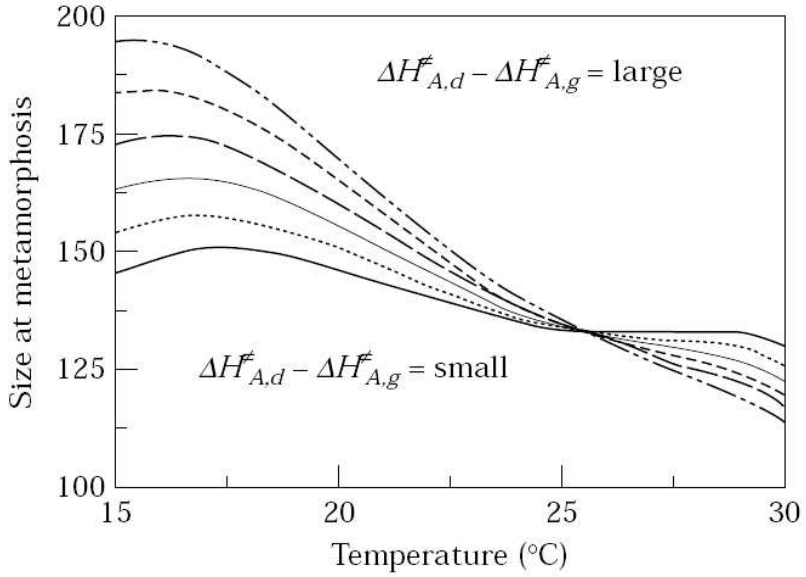

Fig. 5. Size at metamorphosis as a function of temperature [equation (5)] with varying values for $\Delta H^{\neq}_{A,d}$-$\Delta H^{\neq}_{A,g}$: a small difference produces a shallow slope and a large difference leads to a steep slope in the size-temperature reaction norm.

What are the implications for quantitative genetic models for the evolution of reaction norms? First, the biophysical model provides a framework to understand and predict the shape of temperature-induced reaction norms for a variety of life-history characters. Second, it gives

support to the view of reaction norms as continuous and due to allelic sensitivity in expression over environments (Gavrilets, 1986, 1988; Gavrilets & Scheiner, 1993*a,b*; de Jong, 1989, 1990, 1995) as opposed to alternative views based on discrete environments (Via & Lande, 1985, 1987; Via, 1993) or on gene regulation (Schlichting & Pigliucci, 1993; Schlichting & Pigliucci, 1995). It shows that structural and heritable variation in enzyme characteristics can be translated into variation in reaction norm shape, without inferring regulatory genes or other complex explanations. Third, it suggests how genetic variation in the temperature dependence of ectotherm body size could be predicted from genetic variation in the reaction norms of growth rate and differentiation rate. Separating the effects of selection on growth rate and differentiation rate is therefore important in understanding the evolution of complex life-cycles of organisms living in heterogeneous environments (Newman, 1988; Bernardo, 1993, 1994).

Fourth, the biophysical model provides a proximate explanation how genotype by environment interaction in the temperature dependence of life-history characters, such as differentiation rate, body size and egg size, originates in the thermodynamic properties of enzymes. In fact, it is impossible to get parallel reaction norms by varying the enzymatic temperature coefficients. In addition, the biophysical model suggests that reaction norms cross and the additive genetic variance of the character under study becomes minimal at the optimal temperature, that is when enzyme activity is maximal (Figs. 2 & 5). In *Drosophila melanogaster* from Tanzania, Noach *et al*. (in press) found the reaction norms for wing length to cross, giving a minimum in the additive genetic variance, at the environmental temperature the population would be adapted to.

WHY DO ECTOTHERMS BECOME SMALLER AT HIGHER GROWTH TEMPERATURES?

It has been commonly observed that ectotherms under experimentally controlled conditions metamorphose or mature at a smaller size when environmental growth temperatures are higher (*e.g.*, Von Bertalanffy, 1960; Precht *et al.*, 1973; Ratte, 1984; Ray, 1960; Atkinson, 1994*a,b*, and many references cited in these papers). Several studies of *Drosophila melanogaster* suggest that this overall decrease in size is predominantly caused by smaller cells (*e.g.*, Robertson, 1959; Partridge *et al.*, 1995). The biophysical model provides a proximate explanation for this phenomenon: if the activation energy constant of differentiation (mainly depending on DNA replication during the cell division cycle) is higher than of growth (mainly depending on protein synthesis), cells will be smaller after dividing at higher temperatures resulting in a smaller overall organism. Although size reduction at higher growth temperatures in virtually all ectotherms has been termed a "biological law" (Atkinson, 1994*a,b*), few proximate explanations have been put forward.

Von Bertalanffy (1934) defined growth as the net energy surplus of absorption and metabolism. To explain smaller size at higher growth temperatures, he suggested that the temperature coefficient of absorption is much lower than the temperature coefficient of metabolism, as the first depends more on physical processes like permeation and diffusion, and the second being more of a chemical nature (Von Bertalanffy, 1960). The differential effect of temperature on these physiological processes would eventually lead to a smaller final size.

Perrin (1988) formalized von Bertalanffy's argument in a simple mathematical model to explain final or asymptotic body size in a study of the cladoceran *Simocephalus vetulus*. However, Perrin could only explain smaller final size by the empirically found lower "adult" growth rate in his experiments, that is a decreasing growth rate after the start of reproduction.

Again, Perrin's model could not explain the significantly smaller size at first reproduction before the obvious decrease in growth rate, while it was also this size that so obviously decreased with temperature in his experiments (Perrin, 1988). To conclude, it seems unlikely that size at maturation can be adequately explained on the basis of growth rate variation and energy budget alone, as in von Bertalanffy's model, although his argument is also in favour of a lower temperature coefficient of growth compared with differentiation.

PHYSIOLOGICAL AND EVOLUTIONARY TIME-SCALE

Van Straalen (1983) suggested an operational definition of a physiological time-scale (sensu Taylor, 1981). He noted that, if temperatures varies, a common physiological time-scale to different developmental processes can only be applied if (i) these are monotonic functions of temperature and (ii) have identical temperature coefficients. As has been pointed out above, growth rate and differentiation rate vary non-linearly at extreme temperatures according to the Sharpe-Schoolfield equation and may have intrinsically different temperature coefficients. This severely limits the application of physiological time-scales to temperature variation in life-history characters, as is implied by the arguments of van Straalen (1983). One could even conclude that physiological time-scales cannot be applied to reaction norms of life-history characters when temperature varies. Despite this, it is common practice to plot body size variables with developmental time or rate as bivariate reaction norms with varying experimental temperatures (*e.g.*, Gebhardt & Stearns, 1988; Windig, 1994) to correct for differences in developmental rates. If the temperature coefficients of growth and differentiation have not been determined these bivariate plots cannot be interpreted in physiological terms. Furthermore, plotting body size variables against differentiation rate distracts from the proximate mechanism determining size at maturation: the interaction between growth and differentiation. This interaction is a real-time phenomenon, which cannot be simply transformed to some relative, physiological time-scale.

Any change in size at metamorphosis or maturation as a result of a change in developmental timing is by definition heterochrony, irrespective of the time-scale involved (Gould, 1977; McKinney & McNamara, 1991). In these terms the major focus of this paper could be called temperature-induced heterochrony (cf. Emerson *et al.*, 1988). Smith-Gill (1983) first noted the importance of heterochrony in the context of phenotypic plasticity, in particular when environmental factors have a modulating effect on the phenotype. Meyer (1987) applied the concept of heterochrony to understand diet-induced phenotypic plasticity in the cichlid fish *Chichlasoma managuense* and provided a first step towards unifying the concepts of heterochrony and plasticity within evolutionary theory.

CONSTRAINTS AND ADAPTIVE EXPLANATIONS

Adaptive explanations have been formulated, modelled and tested for much of the variation in life-history characters within and among species (Stearns, 1992; Roff, 1992). Consequently, a considerable amount of effort has been put in the search for genetic variation in such characters and their plasticity. Genotypic models of reaction norms are particularly suitable to separate genotypic from environmental effects in continuous environments (Gavrilets & Scheiner, 1993*a*,*b*; de Jong, 1990, 1995) and an increasing number of studies reported genetic variation in reaction norms (*e.g.*, Weis & Gorman, 1990; Sultan & Bazzaz, 1993). On the other hand, there is always the question of which constraints in the developmental program (sensu Oster *et al.*, 1988; Maynard Smith *et al.*, 1985) limit the

response to selection (Scharloo, 1987).

Atkinson (1994*a*,*b*) comprehensively reviewed temperature modulated variation in final size in organisms ranging from plants and protists to ectothermic animals, and concluded that no single overriding explanation could account for the general size reduction at higher growth temperatures. The biophysical model, however, identifies temperature constraints to growth and differentiation and derives the conditions for a size reduction at higher temperatures, although adaptive variation may evolve in most parameters. The conditions (equation 6) are easily fulfilled (see Fig. 5). As a proximate model, the biophysical models applies to all ectotherms, including protists in which "differentiation" consists only of cell divisions. A single explanation is given for size reduction at higher temperatures, independent of the level of environmental variation or life history patterns. A single general adaptive explanation for size reduction and shortening of development time at higher temperatures has as yet eluded formulation for such a wide range of organisms, including protists, plants and ectotherms.

Sibly & Atkinson (1994) attempted to model an adaptive explanation for size reduction at higher temperatures. They started from the observed increase of adult size with higher juvenile growth rate at a single temperature. Their model indicates that size reduction at higher temperatures might be an optimal strategy, but only in temporally variable environments and if juvenile mortality rate increases at higher temperatures. Spatial variation would, however, not lead to size reduction at higher temperatures as an adaptive strategy. While Sibly & Atkinson (1994) succeed in finding some conditions in their life-history model that lead to size reduction and short development time at higher temperatures, their paper clearly shows how difficult it is to find an adaptive explanation that is general enough to explain a virtually universal phenomenon.

The biophysical model might have wider applicability than to development alone, as differentiation and growth also occur in the adult stages, most prominently during reproduction. As has been mentioned above, the thermal limits of reproduction may be dictated by the same processes which limits developmental tolerance limits. Analogous to this argument, it is possible to express propagule size as the result of oocyte differentiation alternating with oocyte growth (vitellogenin synthesis) during oocyte production in ovarioles and oviducts in insects (Ernsting & Isaaks, manuscript). From this assumption the same effect of temperature on size at metamorphosis could be predicted, that is, smaller eggs will be produced at higher environmental temperatures. In fact, a correlation between egg size and environmental temperature has been reported in ectotherms on numerous occasions (*e.g.*, in *D. melanogaster* Avelar, 1993; a carabid beetle Ernsting & Isaaks, manuscript; for an overview see Roff, 1992 p. 386-388). The proximate explanation for temperature dependence of egg size in ectotherms could be seen as a null model against which adaptive explanations of environmental variation in propagule size could be tested (Roff, 1992).

The biophysical model provides a proximate framework to study the constraints of life-history characters in a thermally variable environment. The reaction of both growth and differentiation to temperature is a general increase in rate, that can be considered as a biophysical constraint determined by the Eyring equation (Sharpe & DeMichele, 1977). Only further studies can reveal how many degrees of freedom exist in the thermodynamic properties of protein synthesis or the cell division cycle. With respect to the latter, the considerable increase in understanding the molecular genetics of the cell division cycle in recent years (Murray, 1992; Hartwell & Weinert, 1989) could provide ample opportunity to test these ideas. The interaction between growth and differentiation defines another constraint: an intrinsic difference in the temperature coefficients of differentiation (cell division) and growth (protein synthesis) determines the reaction of ectotherm body size to environmental temperature. That reaction does not have to be of an adaptive nature *per se*. It might be a

perfect example of a spandrel (Gould & Lewontin, 1979), the temperature dependence of growth and differentiation being the structural elements. On the other hand, the interaction between growth and differentiation can easily be put to adaptive use.

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CHAPTER 3

A PROXIMATE MODEL FOR THERMAL TOLERANCE IN ECTOTHERMS

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Abstract

Thermal limits of viable ectotherm development are threshold-like and near-symmetrical around the temperature of optimal performance and usually well within the thermal tolerance range of adult physiological traits. A proximate model is proposed to show that the interaction between reversible temperature inactivation of cell cycle proteins and their regulation can explain (1) the symmetry and (2) threshold character of thermal limits of viable embryonal and larval development in ectotherms. It is suggested that temperature inactivation of regulatory proteins mimics the decrease in concentration resulting from gene dosage change and transcriptional regulation during the cell cycle. If certain regulatory proteins have equal probability to be active or inactive at a certain temperature, cell division and, consequently, development becomes blocked. Model predictions were tested by comparing thermal tolerance limits as observed in viability experiments with 14 developing insect species with the estimated temperatures at which a hypothetical rate-determining developmental enzyme has an equal probability to be active or inactive. These 'expected' thermal limits were derived from the Sharpe-Schoolfield equation which describes temperature-differentiation rate reaction norms. In 21 out of 23 comparisons 'expected' thermal limits agree closely with the observed thermal tolerance limits. The implications of the model for thermal tolerance, thermal adaptation, epidemiology and life-history strategies are discussed.

Keywords: Thermal tolerance limits, viability, development rate, reversible inactivation, cell cycle, insects,

Introduction

In many ectothermic organisms fitness components and, ultimately, realised fitness are usually highly dependent on the thermal environment (Gilchrist 1995). These organisms have to adapt to the limitations imposed by either high temperature, leading to irreversible denaturation of proteins and death, or low temperature, leading to zero activity of proteins by inactivation (Hochachka and Somero 1984). Studies of evolution in thermally variable environments focuses usually on the breadth of tolerance, survival probability or viability as a function of temperature, and performance, such as fecundity, growth rate, and running speed as a function of temperature (Huey and Kingsolver 1989). Evolutionary models suggest that for most types of thermal variation in the environment organisms should specialise in performance and generalise in tolerance (Gilchrist 1995). Quantitative genetic studies, however, albeit few in number are equivocal. The study of thermal sensitivity of parasitisation capacity in an egg parasitoid revealed that an increase in optimum temperature was accompanied by a raise in maximum parasitisation performance (Carrière and Boivin 1997). On the other hand, a quantitative genetic analysis of locomotor performance (in a hymenopteran parasitoid, Gilchrist 1996) and life-history traits (in a fruit fly, Partridge et al. 1995) detected trade-offs between maximum performance and performance breadth.

Understanding the proximate mechanisms determining thermal tolerance breadth is fundamental to thermal ecology and essential to the development of realistic models of the genetics and evolution of thermal sensitivity (Scharloo 1989). An important question in this context is, whether a single mechanism could be responsible for thermal limits at both high and low temperatures. This would be in contrast to the traditional view that at least two different processes are involved, reversible and irreversible inactivation, respectively, at low and high temperatures.

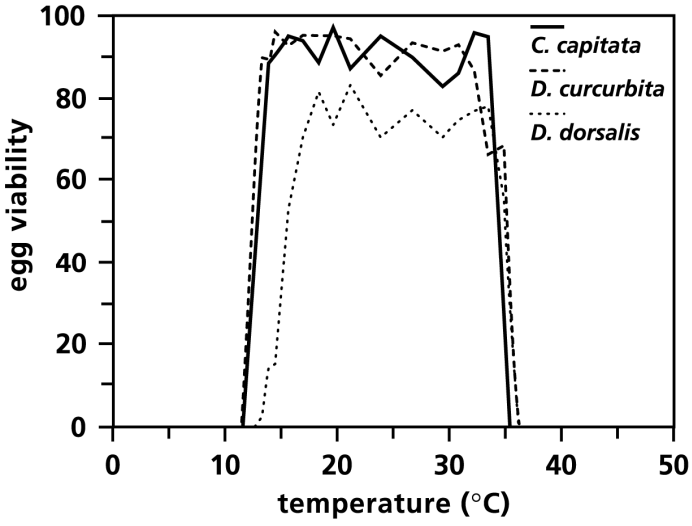

Fig. 1. Temperature viability curves of the fruitflies *Dacus dorsalis, D. cucurbita* and *Ceratitis capitata* illustrates the general pattern of temperature tolerance limits of developing ectotherms. Data from Messenger and Flitters (1958).

Both viability (Fig. 1) and performance curves (Fig. 2a) can be seen as a 'set of phenotypes expressed by a single genotype across a range of environmental conditions', or reaction norms (Lynch and Gabriel 1987, Gabriel and Lynch 1992, Schmalhausen 1949,

Woltereck 1909). Adaptive evolution will select for some optimal reaction norm (Gavrilets and Scheiner, 1993a,b) within the limits set by developmental constraints (Scharloo, 1987). During development there is at least one clear biophysical constraint: most biochemical rates increase with increasing temperature, as determined by the Eyring equation. Each biochemical process has its own specific temperature coefficient, leading to quite different outcomes of physiological and developmental processes at different temperatures (*e.g.*, van der Have and de Jong, 1996).

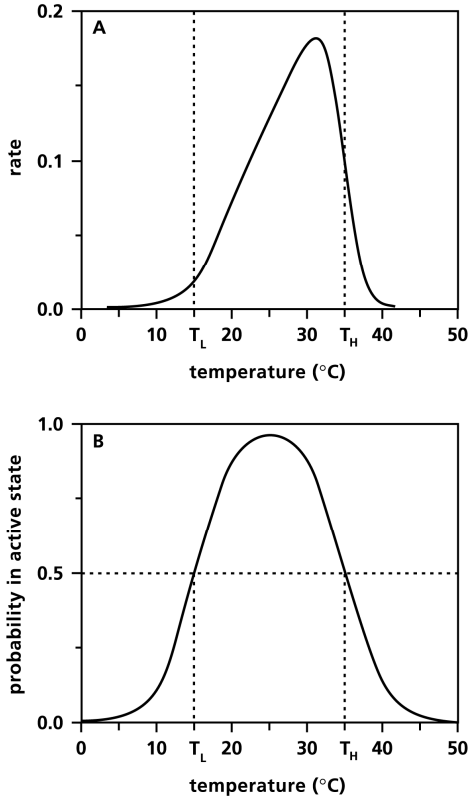

Fig. 2. (A) General shape of the relationship between biological performance (walking speed, heart beat) or rate (division rate, growth rate) and temperature. The developmental tolerance limits are indicated by broken lines. Note that the maximal performance is close to the upper thermal limit (from Huey and Kingsolver 1989). (B) Temperature dependence of the probability of a protein being in active state (from Sharpe and DeMichele 1977). T_L and T_H are the temperatures at which the protein has equal probability to be active or inactive.

For realistic evolutionary and physiological models it is essential to have detailed knowledge of the differential effects of temperature on the mechanisms and genetics involved (Pigliucci, 1996; Schlichting and Pigliucci, 1995; Scharloo, 1989). This implies that it is necessary to know how enzyme activity and regulation result in different phenotypes in different environments (Gavrilets and Scheiner, 1993b). The underlying genetics and mechanisms of reaction norms are not well understood (Pigliucci, 1996), with some notable exceptions such as the temperature dependence of wing spot variation in butterflies (Brakefield et al., 1996).

Temperature-viability reaction norms of ectotherms grown at constant temperatures generally have an inverted u-shape (*e.g.*, insects, Cohet et al. 1980, Messenger and Flitters 1958, amphibians, Bachmann 1969, Fig. 1). The thermal limits of development can be characterized as sharply defined thresholds at high and low temperatures and symmetrical around the median temperature of viability. The permissive temperature range of embryonic development is usually much narrower compared to the tolerance range of adult physiology like respiration, metabolism

in general, or derived performance parameters like running speed or flight speed (fish, Brett 1970; *Drosophila*, David et al. 1983).

This phenomenon is well illustrated by the comparison of thermal limits of embryonic development to the critical thermal limits of adult anurans collected at different latitudes (Fig. 3). The thermal range of adult anurans (63 species, data from Brattstrom 1968) varies from 30 degrees Celsius at tropical latitudes to 40 degrees at temperate latitudes (Snyder and Weathers 1975) and tracks the decrease in the mean and increase in the variance of environmental temperature with latitude. At temperate latitudes the embryonic thermal range (44 species, data from Brown 1975, Moore 1942, Volpe 1957, Zweifel 1968 and references therein) varies between 17 degrees at tropical to 24,5 degrees at temperate latitudes. The embryonic range clearly tracks the decrease in mean environmental temperature, but the data set is inconclusive with respect to the change in environmental variance. Furthermore, adult performance is usually asymmetrical, that is, the maximal performance temperature is close the upper tolerance limit (Fig. 2a, Huey and Kingsolver 1989).

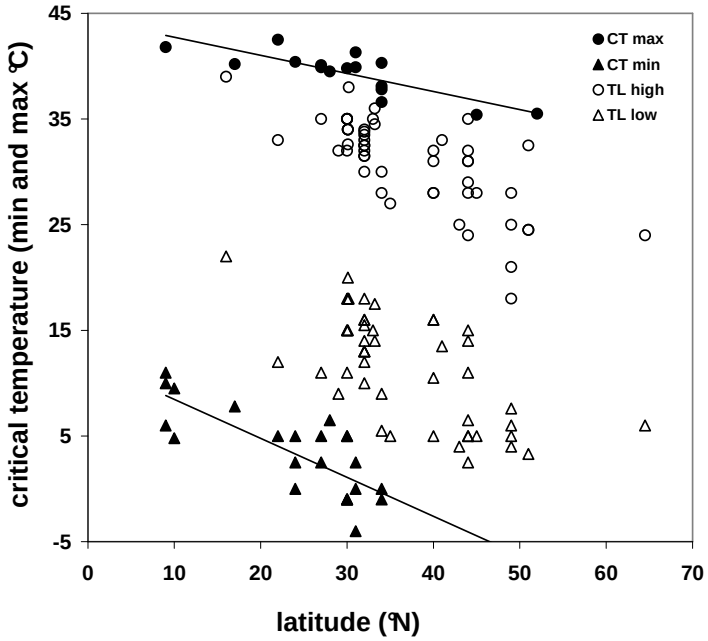

Fig. 3. Latitude and the upper and lower thermal limit of embryonic development (open squares and triangles, respectively) and critical thermal maximum and lower lethal temperature of (post-embryonic) mature anurans (closed circles). Embryonic thermal limits are taken from Brown 1975, Moore 1942, Volpe 1957, Zweifel 1968; critical thermal limits of mature anurans are extracted from Brattstrom 1968 (see Snyder and Weathers 1975 for details on methods and data selection).

To date, few attempts have been made to explain the threshold character or shape of the temperature-viability reaction norms and the difference with adult performance. Differences in thermal performance and thermal limits can have profound effects on the interaction between species (Gilbert and Rawort 1996, Harrington et al. 1999, Davis et al. 1998). The general observation that embryonic and larval thermal tolerance is more limited than adult performance suggests that these life-history stages may be more important mediating the outcome of the interactions than the adult stages.

One obvious difference between the adult stage and the embryonic and larval stage in

ectotherms is the relative intensity of cell division and differentiation. During development most cells are actively dividing, while in the adult stages cell division occurs mainly in regenerating processes and reproductive tissue not directly linked to performance of the whole organism. This suggests that temperature-induced conformational changes of proteins involved in cell cycle regulation may block cell division and by implication determine the thermal limits of development.

It seems unlikely that symmetrical tolerance limits could be explained with irreversible inactivation or denaturation of proteins, which occurs mainly at temperatures higher than the upper thermal limit. The temperature dependence of biochemical reactions and their transition rate constants are usually monotonically increasing functions of temperature described by the Eyring equation (Alexandrov 1977, Sharpe and DeMichele 1977) and cannot be a solution either. On the other hand, reversible inactivation of cell cycle proteins at high and low temperatures could provide a parsimonious explanation as it occurs more or less symmetrically around an optimum temperature (Fig. 2b, Sharpe and DeMichele 1977). However, enzyme activity decreases gradually at higher or lower temperatures and reversible inactivation alone cannot explain the threshold-character of the thermal limits. It is necessary to look at processes specific for development and cell division.

The development of a multicellular organism from zygote to the adult stage proceeds through a series of cell divisions. Cell growth and differentiation are closely co-ordinated with cell division during the larval stage, but are dissociated during embryogenesis. Overall, development can be considered as the interaction between differentiation and growth. Differentiation rate (time^{-1}) is assumed to be primarily determined by the cell division rate (van der Have and de Jong 1996). The functions and interactions of the proteins involved in the regulation of the cell cycle are now understood in great detail (*e.g.*, Murray and Kirschner 1989, Nurse 1990, Murray 1992, 1994, Tyson 1991).

First, a proximate model is presented which shows that temperature inactivation of cell cycle proteins interacts with their regulation and can predict the temperature tolerance limits of ectothermic development. The analysis suggests that reversible temperature inactivation at high and low temperatures has a symmetrical, inhibiting effect on the balance between synthesis and degradation of cell cycle proteins, resulting in sharp thresholds at the high and low temperatures, above and below which the cell cycle becomes blocked. Second, the predictions of the model for the shape of viability-temperature reaction norms are tested with differentiation rate - temperature reaction norms in 14 insect species from literature data. Finally, the ecological and evolutionary implications of these temperature constraints at the cellular level are discussed.

The model

Reversible protein inactivation

Sharpe and DeMichele (1977) developed a stochastic thermodynamic model of poikilotherm development derived from the Eyring equation. It was assumed that the developmental control protein (an enzyme in their case) can exist in two temperature dependent inactivation states as well as an active state. At high and low temperatures the protein undergoes a conformational transition rendering the protein inactive. The transitions between energy states are unimolecular and completely reversible and no transitions take place between the high and low inactive states directly. For an individual enzyme molecule the cumulative probability of being in the three energy states is therefore equal to one. The transitions between states are randomly distributed with a mean transition rate k_i (second^{-1}) specified by the Eyring equation:

$$k_i = \frac{KT}{h} \exp\left((\Delta S_i^{\neq} - \Delta H_i^{\neq} / T) / R \right) \quad (1)$$

where h is Planck's constant, K Boltzmann constant, $\Delta H_i^{\neq}$ the enthalpy of activation, $\Delta S_i^{\neq}$ the entropy of activation, T absolute temperature, and R the gas constant. It is assumed that transitions between energy states have reached steady-state. From these assumptions an equation can be derived for the probability P_a that the protein is in active state:

$$\frac{1}{P_a} = 1 + \exp\left(\frac{\Delta H_L^{\neq}}{R} \left(\frac{1}{T_L} - \frac{1}{T} \right) \right) + \exp\left(\frac{\Delta H_H^{\neq}}{R} \left(\frac{1}{T_H} - \frac{1}{T} \right) \right). \quad (2)$$

where T_L and T_H are the temperatures (°K) at which the protein has equal probability to be active or inactive by low or high temperature inactivation, respectively, $\Delta H^{\neq}_L$ and $\Delta H^{\neq}_H$ (after here H_L and H_H) are the change in enthalpy (J mol^{-1}) associated with respectively low or high temperature inactivation of the enzyme (Sharpe and DeMichele 1977, Schoolfield et al. 1981). Fig. 2b shows the bell-shaped function generated by equation 2, describing the temperature dependence of protein activity. If temperature inactivation of proteins in general would determine the thermal limits, then a similar gradual response in viability would be expected. Comparison of Fig. 1 with Fig. 2b shows that temperature inactivation alone cannot explain the threshold-like thermal limits of development. Developmental tolerance curves (Fig. 1) are inverted U's, a high level between sharply defined limits. Activity curves of proteins and enzymes in particular are usually optimum curves with a gradual decline in activity at low or high temperature (Fig. 2b).

Inverted U-shaped curves could be generated by changing H_L and H_H simultaneously and thereby potentially explaining the difference between developmental (Fig. 1) and adult (Fig. 2b) 'performance' curves. This would imply that all important proteins have different embryonal, larval and adult variants, and are expressed at different stages during development, which seems to be an unlikely phenomenon.

Cell cycle regulation in eukaryotes

Several detailed mathematical models of the cell cycle have been developed (Tyson 1991, Novak and Tyson 1993a,b, Goldbeter 1991, Norel and Augur 1991), which complement the intuitive diagrams and verbal arguments of the more qualitatively minded cell biologists (Maddox 1992, 1994). These quantitative models can quite precisely explain the oscillator phenomena in early embryos and switch mechanisms in growth-controlled cell cycles. These models are based on the interactions between the subunits Cdc2 and cyclin of the heterodimer MPF (Maturation Promoting Factor) and cell cycle enzymes (Wee1, Cdc25, CAK and INH, Novak and Tyson 1993a,b).

It may be possible to work out the temperature dependence of the cell division rate on the basis of the above mentioned theoretical cell cycle models, but it seems unlikely that such model will produce symmetrical tolerance limits as observed in many ectotherms. In other words, it seems unlikely that such models will provide the conditions when cell cycle arrest will occur symmetrically at low and high temperature. As transition rates of all catalytic reactions will depend on temperature following the Eyring equation, cell division rate will gradually increase with temperature generally following the model of Sharpe and DeMichele (1977). As argued in the introduction, it is *a priori* difficult to imagine how this asymmetrical, gradual response can produce symmetrical and sharply defined, threshold-like limits at which the cell

cycle becomes arrested.

Reversible inactivation of cell cycle enzymes will slow cell division down at low temperatures and as well as decrease it at high temperatures. Furthermore, all enzymes involved will be reversibly inactivated and a gradual response of the whole system can be expected, not the switch-like behaviour of the developmental tolerance limits we are pursuing to explain. It cannot be ruled out, though, that such system behaviour could result from reversible enzyme inactivation in more sophisticated cell cycle models involving ten or more coupled, non-linear ordinary differential equations (Novak and Tyson 1993a,b). For the moment it is assumed, that the thermodynamic properties of these cell cycle enzymes with respect to temperature inactivation are similar, although empirical data are currently lacking. Sharpe and DeMichele (1977) provide a graphical argument showing that if the inactivation parameters of the enzymes limiting development rate differ much, the thermal range of development decreases considerably, although the general development rate function will have the same shape.

Therefore, it seems more promising first to look for specific components in cell cycle regulation which could be expected to differ intrinsically in their temperature dependence because of their different biochemical functions. For example, one could compare the thermodynamics of different types of first-order reactions, such as protein-DNA binding with protein-protein binding. These types of reactions are involved in gene regulation, repression and inhibition of the cell cycle components (Harper et al. 1993, Welch and Wang 1993, El-Dreiry et al. 1993, Hengst et al. 1994, Serrano et al. 1993). Alternatively, first-order reactions could be compared with higher order, catalytic reactions. The latter type of reactions are typical of most of the characteristic steps of the cell cycle (Murray and Kirshner 1989, Nurse 1990, Tyson 1991, Novak and Tyson 1993a,b).

A simple model of derepression as a control mechanism for the cell cycle in eukaryotes was developed by Tyson and Sachsenmaier (1979). Their model was specifically developed to explain (a) how nuclear and cell division could be initiated only after replication of the entire genome and (b) ensures that DNA is replicated once and only once during every cell cycle (Sachsenmaier et al. 1972, Tyson and Sachsenmaier 1979). Although this model predates the huge progress in the field of cell cycle research, it has recently become increasingly clear that transcriptional regulation has a central role in changes of cell cycle regulation during development (Edgar and Lehner 1996).

Tyson and Sachsenmaier showed how a genetic control system can account for the periodic synthesis of a mitotic activator by sequential dosage changes of an early-replicated repressor and a late-replicated operon. These dosage changes result in periodic switching of the operon from the derepressed to the repressed state and the activator synthesis respectively off and on at the beginning and end of *S*. Their model is relatively simple and involves both protein-DNA (repressor-operon) and protein-protein (repressor-inducer) binding. It therefore fulfils the above stated prerequisite to serve as a starting point for the analysis of the effects of temperature inactivation on proteins regulating the cell cycle.

The genetic control system is as follows. Protein *R*, coded for by an early-replicating gene G_R, binds to operator region *O* and represses (inhibits) transcription of structural genes G_P and G_A. Inducer *P* inactivates the repressor and thus has a positive feedback on its own rate of production. Protein *A* activates mitosis. The system will exist in either of two states: repressed with very little synthesis of *P* and *A* and derepressed with maximum synthesis of *P* and *A*. G_R doubles at the beginning of *S* phase and the operon region replicates at the end of *S*. Tyson and Sachsenmaier (1979) showed that gene dosage changes could account for periodic switching from the derepressed to the repressed state and *vice versa*. They found that the fraction of operons actively transcribing can be expressed as

$$f = \left[1 + \left(\frac{K_2 R_T}{1 + K_1 P} \right)^n \right]^{-1} \qquad (3)$$

where R_T is the total number of repressor molecules, K_i equilibrium constants, P the concentration of the inducer, and n is the number of repressors binding with the operator. Under steady state conditions the rate of synthesis of R, P and A is exactly balanced by the rate of their degradation.

The steady-state concentration x of the inducer P can be defined as $x = K_1 P/K_2 R_T$ and the rate of inducer degradation as $\varphi = l_1 m_2 K_2 G_R / l_2 m_1 K_1 G_P$, where l_i, m_i are rate constants in the differential equations describing the changes in inducer and repressor concentrations, K_i equilibrium constants and G_i the dosage (i.e. the number per cell) of genes coding for molecular species i. Under steady state conditions the rate of inducer synthesis ($f(x)$) will be exactly balanced by the rate of inducer degradation (φx), both relative to their maximum rate of synthesis, *i.e.* where the curve $f(x)$ intersects the straight line φx the steady-state condition can be found:

$$f(x) = \varphi x. \qquad (4)$$

Since φ is proportional to the ratio of repressor gene dosage to inducer gene dosage, the relative rate of inducer degradation φx doubles abruptly in early S ($G_R \rightarrow 2$), then halves again in late S, as G_P replicates ($G_P \rightarrow 2$). In Fig. 4a it can be seen that the slope φ_S in early S is twice the slope φ_G in late S. The shape of $f(x)$ is always sigmoid, and if appropriate values for the kinetic constants are chosen, this change in slope φ of the degradation rate can switch the operon off ($f(x) \approx \varepsilon^2$), when transcription is negligible, and on ($f(x) \approx 1$) at approximately maximum rate. This is depicted in Fig. 4a, where φ_S and φ_G are represented by two broken lines with different slopes and which intersect with $f(x)$ (continuous line) near the origin and near maximum transcription rate, respectively (Tyson and Sachsenmaier 1979). In the following section we will explore the differential effects of temperature on the components of this genetic control system.

Temperature inactivation of cell cycle proteins

The following simplifying assumptions could be made for the thermodynamics of the regulation of the cell cycle.

i) The inducer and repressor are proteins, which are assumed to occur in three energy states: active or reversibly inactive at high or low temperature (Sharpe and DeMichele 1977). At high and low temperatures these proteins undergo a conformational transition which renders them inactive with respect to binding properties (Somero 1995).

ii) The thermodynamics of the repressor differs from that of the inducer in that the latter is more thermolabile than the former (Polyak et al. 1994), so that the temperature inactivation of the repressor can be ignored over the temperature range at which inactivation of the inducer occurs.

iii) The reversible inactivation of the inducer follows equation (2).

iv) The temperature dependencies of all transition rates l_i, m_i and equilibrium constants K_i are assumed to be similar, i.e. the rate of inducer degradation $\varphi = l_1 m_2 K_2 G_R / l_2 m_1 K_1 G_P$ is temperature-independent.

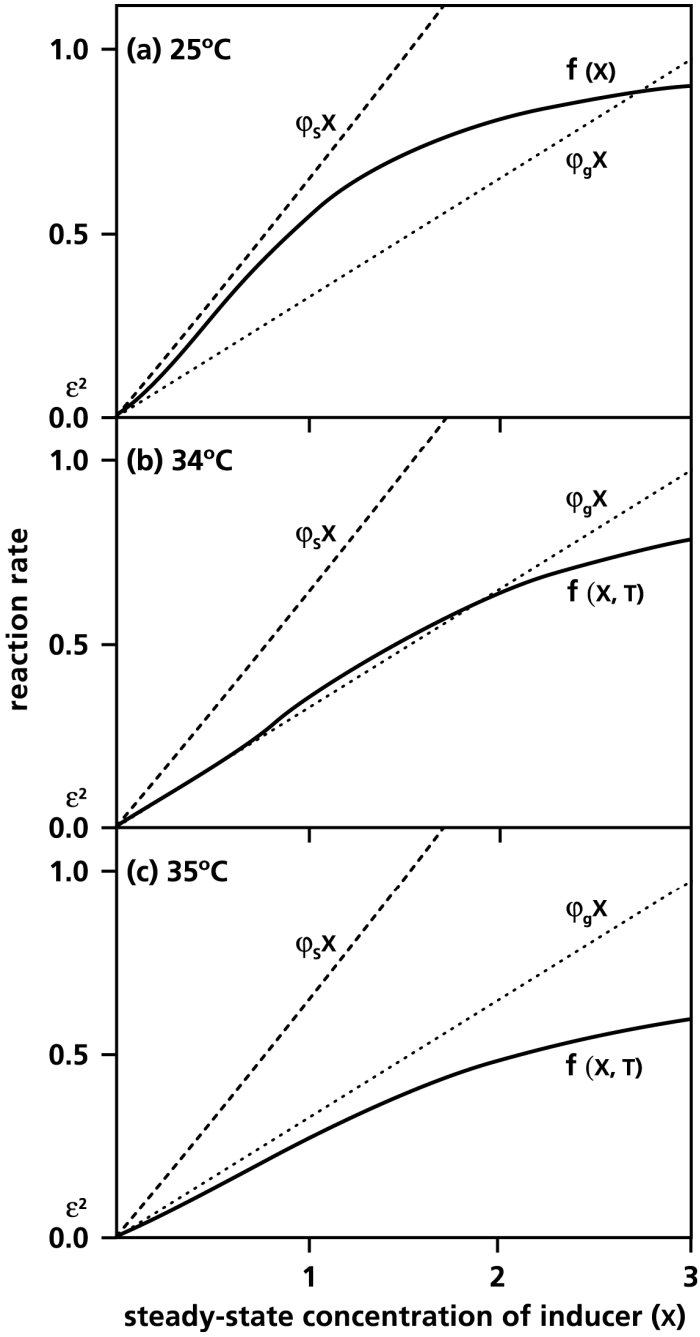

Fig. 4 (a) Reaction rate at steady state concentrations of inducer (x) during *S*-phase (DNA-synthesis) and during the gap phases (*G1* and *G2*) between successive *S* phases without temperature inactivation (25 °C). During *S* the relative rate of inducer degradation (φ_S) is large and transcription from operon is negligible and the intersection between $\varphi_S x$ (broken line) and $f(x)$ (continuous line) is close to zero ($\approx \varepsilon^2$). During *G* phase the relative rate of degradation is reduced ($\varphi_G = \varphi_S/2$) and the operon is transcribed at nearly maximum rate (intersection between $\varphi_G x$ [broken line] and $f(x)$ close to 1). (b) $f(x, T)$ (continuous line) at 34 °C and (c) at 35 °C. See the text for a further explanation.

From these assumptions it follows that (a) the temperature-dependent probability P_a that the inducer is in active state can be described by equation (2), and (b) the effective inducer concentration becomes P_aP at temperature T. Then, the temperature dependence of the fraction of transcribing operons becomes

$$f(x,T) = \left[1 + \left(\frac{K_2 R_T}{1 + K_1 P_a P}\right)\right]^{-1} . \qquad (5)$$

The relative rate of inducer degradation φx remains unchanged within the temperature range under consideration, and doubling of φx in early S would still happen (broken lines in Fig. 4b, c) even if the constants are temperature-sensitive, because the temperature dependence of all transition rates and equilibrium constants is assumed to be similar. If equation (2) is substituted in (5), the temperature dependence of the steady state concentration of inducer during DNA-synthesis and during the gap-phase can be derived. This can illustrated by a numerical example. For P_a a set of constants is chosen based on values estimated for a range of poikilotherm organisms: H_L= -70.000 and H_H= 70.000 (Sharpe and DeMichele 1977). The temperatures T_H and T_L, at which the protein has equal chance to be active or inactive by high or low temperatures, respectively, are chosen at 288 °K (15 °C) and 308 °K (35 °C).

If the temperature is increased from 25 °C to 34 °C, $f(x,T)$ gradually flattens, the intersection of $f(x,T)$ with $\varphi_S x$ at $f(x,T) \approx \varepsilon^2$ remains the same, while the intersection of $f(x,T)$ and $\varphi_G x$ is at a slightly lower value of x (Fig. 4a, b). If the temperature is increased further the operon transcription rate decreases, but at certain temperatures two or three equilibria during G are possible as can be seen from the number of intersections of $f(x,T)$ with $\varphi_G x$. At 35 °C only one equilibrium near the origin remains (Fig. 4c). Between 34 °C and 35 °C (T_H) a sharp change in the point of intersection occurs, from about the highest to a negligible value of x (Fig. 4b, c). At decreasing temperatures a similar switch occurs between 14 °C and 15 °C (T_L) for the chosen parameter values. These parameter values do matter, because one, two or three intersections of $f(x,T)$ with $\varphi_G x$ are possible when temperature increases or decreases. However, if ε is not too small multiple equilibria occur only in a very small temperature range between 34 °C and 35 °C.

From Fig. 4 it becomes clear that the 'window' between $\varphi_S x$ and $\varphi_G x$ determines the temperature range of a functional cell cycle, outside which the operon is switched off by reversible temperature inactivation. This temperature range is widest if the kinetic constants are chosen such that the quasi-linear section of $f(x,T)$ falls close to $\varphi_S x$ (Fig. 4a). The intersections between $f(x,T)$ and $\varphi_G x$ can be found numerically and the temperature dependence of the operon transcription rate has an inverted U-shape, if only the intersection is used with the highest value for x (Fig. 5). At intermediate temperatures the operon is transcribed near maximum rate. If the kinetic constants are chosen such that $f(x,T)$ falls close to $\varphi_S x$ at 25 °C, the operon is switched off ($f(x,T) \approx \varepsilon^2$) at the temperatures at which the inducer has equal chance to be active or inactive (equivalent to T_H and T_L , Fig. 5). This implies that DNA-replication and as a result cell division will become (reversibly) blocked at the temperature at which the inducer is only half active, while the potential range of biological activity is much wider. Temperature inactivation of the inducer, therefore, mimics the decrease in inducer concentration resulting from gene dosage changes during the cell cycle.

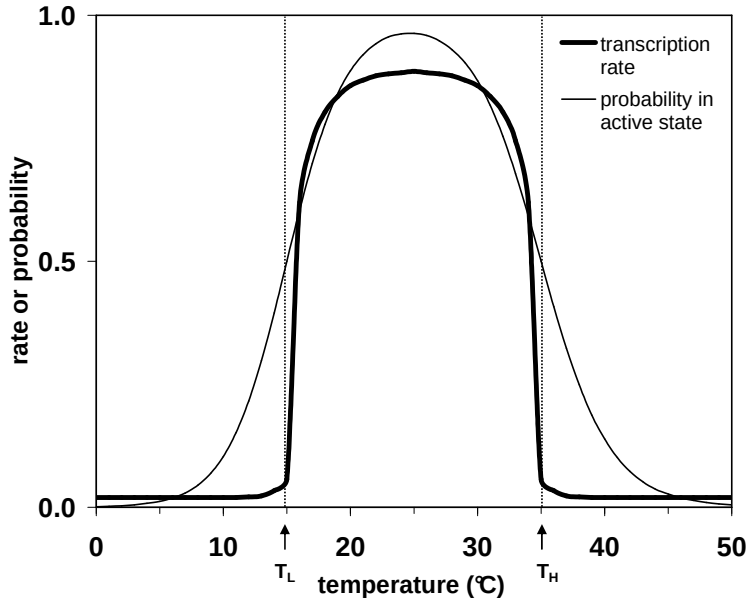

Fig. 5. The probability that the inducer is in active state (P_a) (thick continuous line) and the transcription rate of the genetic operon $f(x,T)$ (thin continous line) at different temperatures. A sharp decrease from near maximum transcription rate to $f(x,T) \approx \varepsilon^2$ to zero transcription rate occurs at temperatures when the inducer (rate-limiting developmental protein) is approximately half active and half inactive (broken line). As a result, cell division becomes blocked and development becomes impossible at the lower (T_L) and upper (T_H) thermal limit.

Implications

Thermal tolerance is a central issue in the thermobiology of ectotherms and is supposed to be a target of selection and adaptation (Huey and Kingsolver 1989). The focus is usually on the performance of adult traits, such as running speed, metabolic rate or heart rate, and the thermal limits are generally thought to define the thermal niche. Physiological traits depend broadly on metabolic efficiency and metabolic pathways, which are not directly linked to the cell cycle or DNA-replication. The fact that the thermal limits of viable ectotherm development (between 10 - 35 °C) are often much narrower than of the performance of adult physiological traits (between 0 - 50 °C) is widely known but its possible cause has been generally disregarded. The current model suggests an explanation for the observation that the performance breadth of development is usually narrower than adult performance. Consequently, studies that are limited to adults might give a biased view of thermal tolerance, in particular as immature stages of terrestrial ectotherms are often less mobile than the adult stages.

The proximate model presented here suggests that inactivation of the regulatory proteins involved in first-order reactions could be responsible for the threshold character of the thermal limits, instead of a gradual change in viability. It is likely that the thermal characteristics of the cell cycle enzymes do not differ much if no large functional differences exists, like the tight binding with DNA of certain inhibitors. Selection for maximal development rate at a certain temperature will generally select for enzymes highly efficient at this temperature, which is traded off against the width of the thermal tolerance range (Somero 1995).

The threshold-like thermal limits of ectothermic development resemble the phenotypic effects of temperature-sensitive cell cycle mutants (*e.g.*, Nurse 1990), that is the mutant phenotypes are expressed above or below a certain temperature without any gradual change. These effects have been used to grow large-celled yeast strains, when cell growth continues at temperatures non-permissive for cell division. This implies that increased cell size and perhaps increased overall size of ectotherms are expected if experimental temperatures during

development are alternated below and above the thermal limits, temporarily inhibiting cell division but not growth.

If the first-order reactions between inhibitors and cyclin-CDK's are rate-limiting to the cell division rate and by implication to differentiation rate (*e.g.*, Sharpe and DeMichele 1977), then the thermal limits of ectotherm development might be expected to be exactly at the temperatures where the developmental enzyme is half active and half inactive (Schoolfield et al. 1981). This prediction can be tested by estimating thermodynamic parameters from temperature - differentiation rate reaction norms with the Sharpe-Schoolfield equation.

Reaction norms in ectotherms

Sharpe and DeMichele (1977) derived a biophysical model to describe the temperature dependence of any aspect of poikilotherm development, such as differentiation rate, cell division rate or growth rate. Their model is derived from Johnson and Lewin (1946), and in its basic form already proposed by Briggs and Haldane (1925). The model is based on the thermodynamic properties of a system acting as a single, hypothetical, developmental enzyme which is rate-limiting to development.

This developmental enzyme is assumed to be characterized by a constant molecular population which exists either in active form (at normal temperatures) or in reversibly inactive forms (at high or low temperatures). The biophysical model differs in this respect from the 'thermal performance', where only the decrease in reaction rates at higher temperatures is linked with thermal instabilities of enzymes (Hochachka and Somero, 1984; Heinrich, 1977). The Sharpe-Schoolfield model includes inactivation at both low and high temperature.

By combining the Eyring equation (1) with reaction rate kinetics Sharpe & DeMichele (1977) and Schoolfield *et al.* (1981) derived an equation (the Sharpe-Schoolfield equation) for any rate of development under non-limiting substrate conditions:

$$r(T) = \frac{\rho T P_a}{298.2} \exp\left(\frac{H_A}{R} \left(\frac{1}{298.2} - \frac{1}{T} \right) \right) \qquad (6)$$

where $r(T)$ is the mean differentiation rate ($days^{-1}$) at temperature T (°K), P_a is the probability that the rate controlling enzyme (of growth or differentiation) is in an active state as defined by equation (2), R is the universal gas constant (8.314 $J\ K^{-1}\ mol^{-1}$) and 298.2 is a standard reference temperature in degrees Kelvin (equivalent to 25 °C). The thermodynamic parameters are as follows: ρ is the differentiation rate ($days^{-1}$) at the standard reference temperature of 25 °C assuming no enzyme inactivation (this implies that 25 °C is the optimal temperature), H_A is the enthalpy of activation ($J\ mol^{-1}$) of the rate controlling enzyme.

The Sharpe-Schoolfield equation has considerable advantage over other expressions of biological rate functions (reviewed in Wagner et al. (1984b). It can accurately describe the temperature dependence of a developmental process over the total range of biological activity, including the quasi-linear region at intermediate temperatures and the non-linear regions at high and low temperatures (Fig. 2a). Reversible inactivation of enzymes approximately linearizes the exponential rate function expected from the Eyring equation. It should be noted that the maximum rate occurs well above the optimum temperature of 25 °C, which is defined as the temperature at which no inactivation of the hypothetical developmental enzyme occurs.

The six thermodynamic parameters of Sharpe-Schoolfield equation were estimated with nonlinear regression (Marquardt algorithm SAS, 1988). Starting values for the *Drosophila* species were derived graphically as described in Schoolfield et al. (1981) and the algorithm converged in fewer that 50 iterations in all four species. It was assumed that no high and low inactivation occurs at 25 °C. For the species involved this is a reasonable assumption

as none is specifically adapted to very high (>30 °C) or low (20 °C) temperatures.

Published datasets of differentiation rates and viability of 14 insect species were used to estimate the thermodynamic parameters from the differentiation rate reaction norm and to compare these with observed viability curves. An important condition was that the experimental temperatures should cover the full range of viable development (the whole thermal window) for both differentiation rate (embryonal and/or larval) and viability (embryonal or egg-to-adult). The datasets which fulfilled these conditions included eight species of *Drosophila* (Cohet *et al*., 1980; Gibert and De Jong, 2001), three species of *Dacus* fruitflies (Messenger and Flitters, 1958), the southern pine beetle *Dendroctonus frontalis* (Wagner *et al*., 1984a), and two Homoptera [aphids] *Myzus persicae* and *Lipaphis erysimi* (Liu and Meng, 1989).

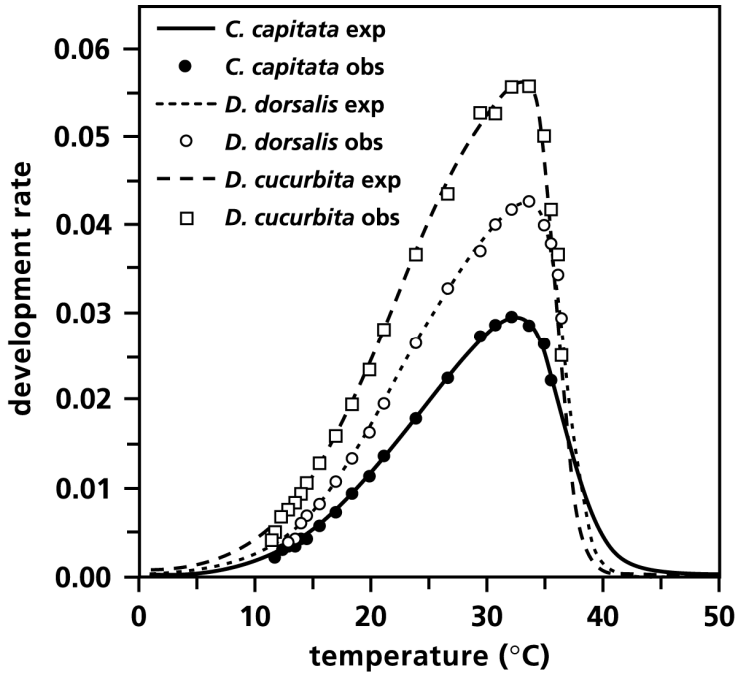

Fig. 6. The temperature dependence of embryonal differentiation rate predicted with the Sharpe-Schoolfield equation and the observed rates in *Ceratitis capitata*, *Dacus cucurbita* and *D. dorsalis* (data from Messenger & Flitters 1958).

Temperature - differentiation rate reaction norms

In all 14 insect species differentiation rate increased with increasing temperature almost linearly up to a maximum and decreased sharply (see for example Figs. 6-7) at higher temperatures. The thermodynamic parameters estimated with non-linear regression applying the Sharpe-Schoolfield equation showed considerable variation in H_A and ρ between species (Table 1). The extremely low values for H_A in *Dacus dorsalis* and *Lipaphis erysimi* are probably unrealistically low, despite the overall good fit. Between the *Dacus* species ρ varied twofold and H_A twofold (excluding the above mentioned outlier), while H_L varied little and H_H varied threefold. Within the *Drosophila* species ρ and H_A varied little and most variation occurred in H_L (fourfold) and H_H (fourfold). The overall fit between observed development rates and the values expected from the Sharpe-Schoolfield equation was in all species very high (Figs 6 - 7) and the six-parameter model could be fitted in all but one (*Drosophila iri*) species.

Table 1. Thermodynamic parameters of the temperature dependence of differentiation rate estimated with non-linear regression and the Sharpe-Schoolfield equation in 14 insect species. Outliers are indicated within brackets.

Species	ρ (10^{-2} hours^{-1})	H_A (kJ mol^{-1})	H_L (kJ mol^{-1})	H_H (kJ mol^{-1})	T_L (°C)	T_H (°C)
Ceratitis capitata[1]	0,088	82	-270	25	14,1	35,4
Dacus cucurbita[1]	0,163	61	-235	302	14,6	36,7
Dacus dorsalis[1]	0,158	(7)	-171	889	22,9	38,9
Drosophila ananassae[2]	0,338	80		342		31,7
Drosophila willistoni[2]	0,326	86		339		29,1
Drosophila subobscura[2]	0,299	99		261		26,4
Drosophila funebris[2]	0,538	111		183		21,4
Drosophila iri[3]	0,488	94		218		32,6
Drosophila yakuba[3]	0,671	86	-1181	225	14,4	32,1
Drosophila simulans[3]	0,550	73	-334	440	13,0	33,1
Drosophila melanogaster[3]	0,596	86	-228	194	12,3	32,4
Dendroctonus frontalis[4]	0,944	66	-430	355	11,5	34,5
Lipaphis erysimi[5]	0,896	(6)	-148	346	18,8	38,3
Myzus persicae[5]	0,871	54	-193	304	8,3	32,3

Sources: [1] Liu *et al*., 1995; [2] Gibert and de Jong, 2001, [3] Cohet *et al*., 1980; [4] Wagner *et al*., 1983a; [5] Liu & Meng, 1989

Temperature - viability reaction norms

All species showed the characteristic inverted U-shaped temperature - viability curve (Figs 8 - 9) and in most species the change from near maximal to zero viability occurred over temperature ranges of only two to three degrees Celsius. The viability curves are compared with the graphs of probability P that the developmental enzyme is in active state (or inactivation curve) estimated with the Sharpe-Schoolfield equation from the temperature - differentiation rate relationships (Table 1). The probability curves could be considered as 'expected' viability curves if viability would directly follow the activity range of the hypothetical developmental enzyme. In most comparisons it is clear that the shapes of the observed viability curves show little resemblance to the 'expected' inactivation curves. The temperature inactivation of the hypothetical developmental enzyme is a gradual response in all cases, except for *Drosophila yakuba* at low temperature limit.

However, if the estimated temperatures at which the developmental enzyme has equal probability to be active or inactive at low and high temperatures (T_L and T_H) are compared with the observed thermal limits, 23 out of 25 comparisons (92%) fall closely together (Figs 8 - 10). The correspondence at high temperatures is remarkably close in all species. The observed lower tolerance limits (T_L) in *Dacus dorsalis* and *Lipaphis erysimi* do not agree with the observed lower thermal limits, but it should be noted that in these species the estimates for H_A were also outliers.

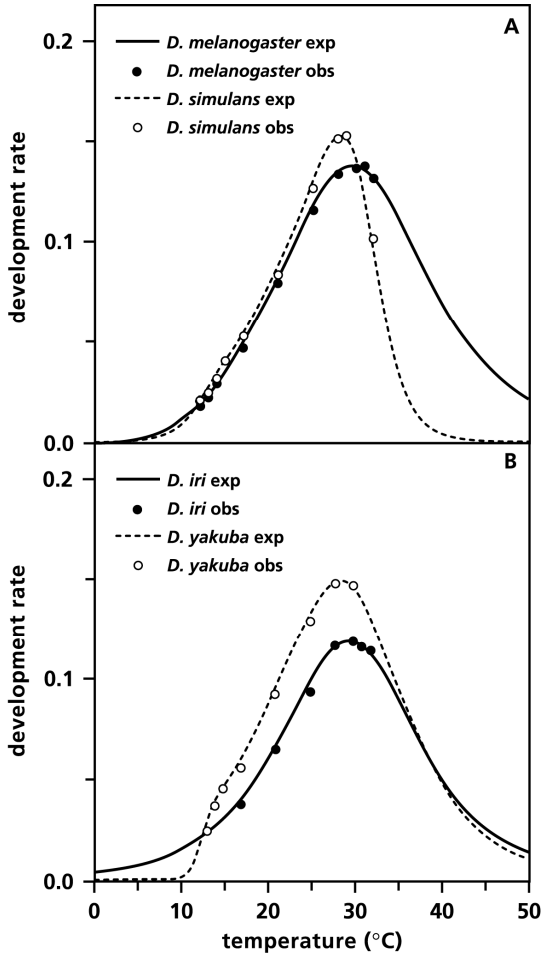

Fig. 7. The temperature dependence of larval differentiation rate predicted with the Sharpe-Schoolfield equation and the observed rates in (A) *Drosophila melanogaster* and *D. simulans* and (B) *D. yakuba* and *D. iri* (data from Cohet *et al*., 1980).

Discussion

Thermal tolerance is a central issue in the thermobiology of ectotherms and a target of selection and adaptation (Huey and Kingsolver 1989). The focus is usually on the performance of adult traits, such as running speed, metabolic rate or heart rate, and the thermal limits are generally thought to define the thermal niche. Therefore, insight in the mechanisms determining the thermal niche is relevant to ecology and necessary to understand the effects of temperature on geographical distribution, population growth, interactions between hosts and parasites, predators and prey, plants and herbivores, among others.
The fact that the thermal range of viable ectotherm development (usually 20 - 25 degrees within the range 10 - 35 °C) is often much narrower, nearly half as wide, than of the performance of adult physiological traits (usually 35 - 45 degrees within the range 0 - 50 °C, see also Fig. 3) is widely known but its possible cause and ecological implications has not received much attention yet. The proximate model presented here suggests that reversible inactivation of the regulatory cell cycle proteins involved in first-order reactions could be responsible for the threshold character of the thermal limits and determines the narrow thermal window of development, instead of a gradual change in viability and wide thermal performance range. In addition, physiological traits depend broadly on metabolic efficiency and metabolic pathways, which are not directly or tightly linked to the cell cycle or DNA-replication.

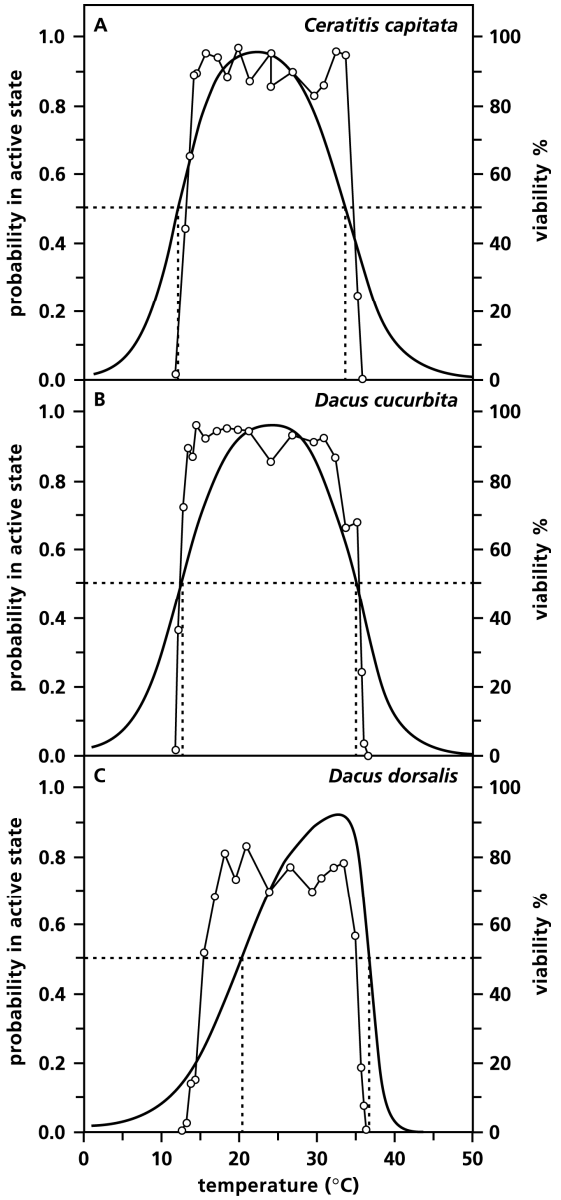

Fig. 8. The temperature dependence of embryonal viability in (A) *Ceratitis capitata*, (B) *Dacus cucurbita* and (C) *D. dorsalis* (line with markers) (data from Messenger & Flitters, 1958). The line without markers represents the probability that the developmental enzyme is in active state according to the Sharpe-Schoolfield equation (parameters estimated with non-linear regression, see text).

First, the assumptions, predictions and tests of the reversible inactivation model will be discussed. Second, the relevance of the Sharpe-Schoolfield equation and the reversible inactivation model to thermal ecology, such as day-degree analysis, host-parasite interactions and variation in performance among different life-history stages will be outlined. Finally, some implications for thermal adaptation and evolution will be suggested.

Model assumptions

The proximate model is rather simple compared to the complexity revealed by recent advances in the understanding of the cell cycle and DNA replication and too general to specify the different postulated proteins. Two assumptions of the model are important: the differential effect

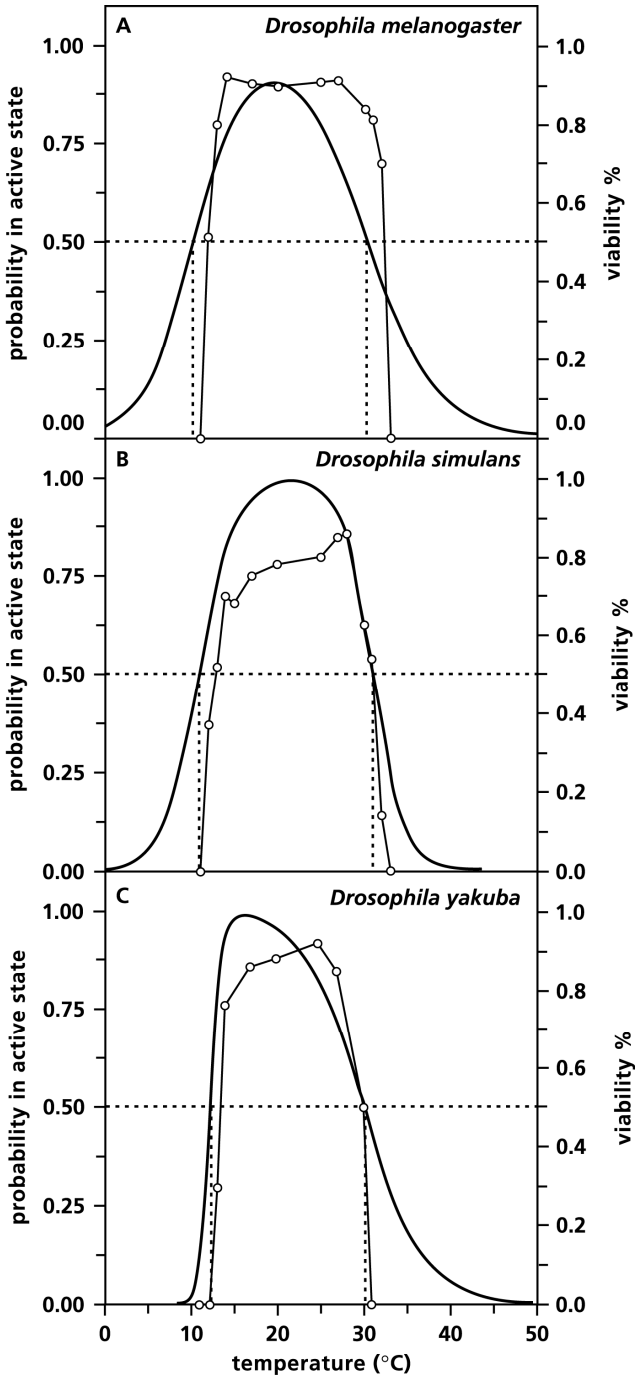

Fig. 9. The temperature dependence of egg-to-adult viability in (A) *Drosophila melanogaster*, (B) *D. simulans* and (C) *D. yakuba* (data from Cohet *et al*., 1980). The line without markers represents the probability that the developmental enzyme is in active state according to the Sharpe-Schoolfield equation (parameters estimated with non-linear regression, see text).

of temperature on reversible inactivation of proteins involved in first-order reactions and dosage change of early and late replicating regulatory genes. The first aspect is supported by the findings of Polyak et al. (1994), who showed that one of the cyclin-dependent kinases responsible for the temporal order of the eukaryotic cell cycle, cyclin E/Cdk2, is inhibited by the heat-stable protein $p27^{KIP1}$. The latter protein is inhibited by a heat-labile binding protein, possibly D-cyclin/CDK6. Cyclin E/Cdk2 is only expressed during *S*, which could be

accounted for by the model of Tyson and Sachsenmaier if the replication order of repressor and inducer is reversed. The thermodynamic difference between inhibitors such as $p27^{KIP1}$ and the cyclin-dependent kinases is likely related to their functional differences within the cell cycle.

The second assumption is supported by recent developments in the molecular biology of DNA-replication which show that both replication and transcription are closely controlled in time as origin of replications are initiated early or late during S-phase by licensing factors and prevented from replicating twice in the same cell cycle (Cardoso et al. 1993, Blow and Laskey 1988, Leno et al. 1992, Fangman and Brewer 1992, Murray 1994, Moreno and Nurse 1994, Diller and Raghuraman 1994). Furthermore, cell cycle proteins (CDC6 and CDC46) were implicated as licensing factors which bind with DNA at the initiation complex (Diller and Raghuraman 1994, Stillman 1996).

Body size and temperature-sensitive mutants

The differential effect of temperature on growth and differentiation has been suggested as a proximate explanation for the general inverse relationship between growth temperature and final body size (van der Have and de Jong 1996). Among evolutionary explanations from life-history theory, increased daily mortality at higher temperatures may explaining size-reduction at increased temperatures (Sibly and Atkinson 1994).

Many cell cycle proteins and their function have been discovered with the use of temperature-sensitive mutants in particular of yeast (*e.g.*, Nurse 1990). At the non-permissive temperature the mutant strains usually arrest in specific stages of the cell cycle, while developing normal at permissive temperatures, suggesting that the thermodynamics of one component may determine the temperature dependence of the whole system, as assumed for example by Sharpe and DeMichele (1977). Apparently, no alternative pathways are known for the cell cycle in contrast with metabolic pathways, although several components may be redundantly present within the cell. A temperature-sensitive eye colour mutant in *Drosophila melanogaster* has been used to determine the thermal niche in the field (Jones et al. 1987). In reverse, the importance of the thermal niche in community structure and competition could be tested by using temperature-sensitive mutants with a much smaller viable temperature range than the wild type.

Model predictions

The Sharpe-Schoolfield equation is a good descriptor of the temperature - differentiation rate reaction norms in ectotherms (Wagner et al., 1984b; Van der Have and De Jong, 1996). The method has been applied to a variety of insect species and the explained variance is usually very high (Wagner et al., 1984b). Furthermore, it is well rooted in biophysical theory of enzyme thermodynamics, which is of considerable advantage compared to other nonlinear equations proposed for the temperature dependence of differentiation rate (see review in Wagner et al., 1984b). The range of activation enthalpies (H_A) of differentiation rate in the 14 insect species is in agreement with values found in a variety of organisms and different processes and fall within the range of 29 - 111 J $degree^{-1}$ mol^{-1} (Kooijman, 1993, Van Straalen 1994, Van Straalen and van Diepen 1995). The low values for *D. d orsalis* and *L. erysimi* are exceptional for unknown reasons.

Despite the realistic values for H_A and close concordance between observed and expected differentiation rates, the temperature - viability curves do not resemble the gradual inactivation curves of developmental enzymes. In addition, thermal tolerance ranges of adult stages are usually wider than tolerance limits during development. For example, heat shock proteins in *Drosophila* are induced at temperatures over 36 °C and adult flies are still active at temperatures below 12 °C and well over 32 °C (*e.g.*, temperature range of mating is 6 – 34 °C (Schnebel and Grossfield, 1984), while development cannot be completed at these

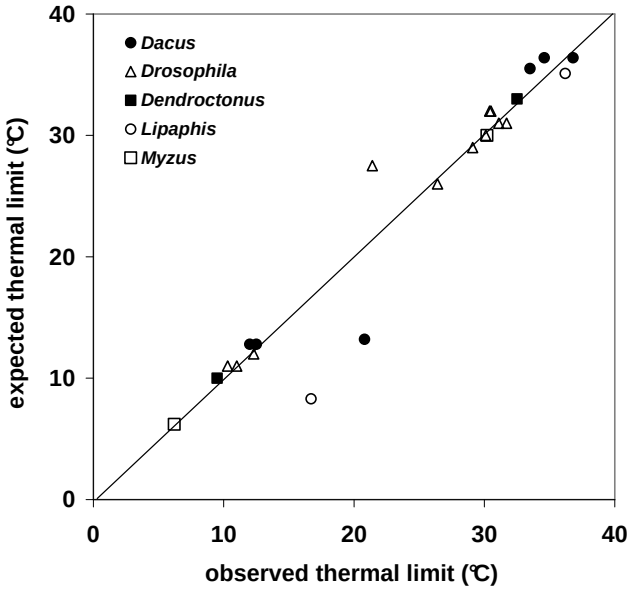

Fig. 10. Comparison of observed thermal limits of viability with expected thermal limits of development, T_L and T_H, in 14 insect species (*Ceratitis*, *Dacus*, *Dendroctonus*, *Drosophila*, *Lipaphis* and *Myzus*) estimated with the Sharpe-Schoolfield equation. The equality line ($y=x$) is drawn.

temperatures (David et al., 1983). The thermal tolerance range in fish becomes wider during growth (Brett 1970). Critical thermal maxima in amphibians are usually in the range 5 – 40 °C (Snyder and Weathers, 1975). Amphibians (and walking insects) are capable of being active over a range of 30 °C (Brattstrom, 1968), while development is usually only permitted within a 20 °C window (Moore, 1942, 1949). These differences suggest that the intensity of cell division during development in the embryonic and larval stages is much higher than in the adult stage.

The overall fit of the predicted thermal limits with the observed ones supports the model for reversible inactivation of cell cycle regulation. It should be noted that the Sharpe-Schoolfield equation is based on one hypothetical, rate-limiting developmental enzyme. The values resulting from nonlinear regression could refer to one rate-limiting enzyme, but it is also possible that it refers to the characteristics of a set of developmental enzymes. Sharpe and DeMichele (1977) showed that characteristics of the multi-enzyme cases are not fundamentally different from the case with one rate-limiting enzyme.

Differential expression of isozymes between life stages could be an alternative explanation among others. If during development the inactivation parameters H_H and H_L, are increased and decreased, respectively, the inactivation curves become more rectangular and similar to the inverted U-shape of viability during the egg, larval or pupal stages. Such a large difference in thermodynamic properties of enzymes in the immature and adult stages could only be acquired by differential expression of many enzymes. Differential expression of isozymes at different temperatures and life stages is a well known phenomenon (Hochachka and Somero, 1984). However, a redundancy of isozymes, of which the activity is limited to extreme temperatures or alternative life stages and maintained by, for example, gene duplication, would incur significant costs (Heinrich, 1977).

A more direct test of the model would be to estimate thermodynamic parameters of cell cycle regulatory proteins of species with differing thermal limits and including their temperature-

sensitive mutants. *Drosophila* and *Xenopus* are both studied intensively in developmental genetics and would be excellent model organisms.

Maximal performance – thermal range trade-off

Infection by a pathogen usually triggers a temperature rise or fever, which may be physiological in endotherms and behavioural in many ectotherms (Kluger 1979, 1991). The current explanation is that the nutrient requirement of the pathogen is elevated at higher temperatures, but the host simultaneously withdraws serum iron and starves the pathogen (Kluger 1979, 1991). In addition, at febrile temperatures the immune response of the host is facilitated (Kluger 1979, 1991). This hypothesis still leaves the question open why the pathogen cannot adapt to the elevated temperatures and limit its nutrient requirements at higher temperatures. This may well be explained by the trade-off between activity range and maximal performance of enzymes (Hochachka and Somero 1984, Somero 1995). Unicellular parasites, in particular, depend highly on cell division for proliferation in the host. By increasing body temperature the host may drive the parasite to its upper thermal limit and and as a result its division rate will remain the same or even decrease (Williams and Nesse 1995, Fig. 2a). This effect gives the host more time to develop an immune response. Lowering body temperature would negatively affect physiological performance essential for escaping predators, finding food or other factors. A stable or decreasing division rate of blood parasites at febrile temperatures has been repeatedly observed (Kluger 1979, 1991). The trade-off between performance and activity range of enzymes and selection for maximum division rate makes it difficult for the unicellular parasite to evolve a wider temperature tolerance range as imposed by the fever response of the host.

Variation among life-history stages

Ecological studies that are limited to adults might give a biased view of thermal tolerance, in particular as immature stages of terrestrial ectotherms are often less mobile than the adult stages. If smaller thermal ranges are always smaller in younger life-stages, seasonal and climatic temperature changes will affect population dynamics. Smaller thermal ranges of developing organisms suggest that thermal (habitat) selection before and during oviposition is critical and implies a narrow thermal niche and high selectivity during this stage. One prediction that follows from the Sharpe-Schoolfield equation would be that the thermal niche of all life stages would be close to the temperature at which the enzymes and regulatory proteins have maximum activity.

Application of the Sharpe-Schoolfield equation and reversible inactivation model will ease the development of predictive models and give insight in the thermodynamics of growth, differentiation and adult metabolism. In particular the trade-off between maximal performance and thermal range is an important issue. Adaption to a common thermal environment may well be different for hosts or parasites, prey or predator, or among competitors and related to their specific life history. This implies that seasonal and climatic temperature change will have very different effects on the outcome of species interactions, such as parasitism, predation, competition and others.

Reversible inactivation of proteins can be a parsimonious explanation for thermal limits in other life stages. The position and shape of the tolerance curve near the thermal limits will depend on the physiological process involved. Upper and lower critical limits of adult viability and performance are often gradual less clear cut than embryonic limits and have to be determined by LD_{50} experiments (*e.g.*, Brattstrom 1968). This pattern conforms more to gradual reversible inactivation of proteins, and enzymes in particular. The reversible inactivation model then implies also that the shape of the upper thermal limit is similar to lower thermal limit and selection on one thermal limit may lead to a correlated response at the other.

Switch-like thresholds for low and high critical temperatures in adult metabolism has been shown in several invertebrates and fish and are often characterized by the onset of

anaerobic metabolism (Sommer et al. 1997). These phenomena suggest that specific steps in metabolic regulation may also involve non-linear effects inducing switch-like patterns when temperature changes. The model presented in this paper would suggest that a functional asymmetry in the thermolability of regulatory proteins may be responsible for the non-linear effects of temperature.

Thermal adaptation and evolution

The phenomenon that a single, inactive enzyme or regulatory protein in the cell cycle can block cell division implies that the temperature characteristics of all proteins involved in the cell cycle need to be closely integrated to enable development over a suitable and wide temperature range (cf. Hochachka 1991). As a result, selection on thermal limits can expected to be more difficult than selection on the thermal sensitivity (the slope of the performance curve, Fig. 2a), as many loci will be involved instead of a few which are rate-limiting. Although the trade-off between thermal sensitivity (and by implication maximal performance) and thermal tolerance breadth is well known at the enzyme level, it is still unclear how evident this phenomenon is expressed at the organism level. Studies which link enzyme performance with phenotypic, whole organism performance are mainly limited to metabolism and metabolic enzymes (Powers et al. 1991, Crawford & Powers 1992).

A central issue in understanding the evolution of reaction norms is the identification of constraints imposed by physiology and biochemistry from an adaptive response to selection (Gotthard and Nylin 1995, Pigliucci 1996, Scharloo 1987, Schlichting and Pigliucci 1995). The molecular mechanisms suggested above may be helpful in this respect. At least, it seems clear that the switch-like shape of the viability curves near the thermal limits is a direct consequence of a proximate mechanism (cell cycle regulation) and not directly shaped by natural selection. The exotic diversity of regulatory proteins involved in the cell cycle is currently extended rapidly by molecular biologists. This active field of research provides an overwhelming amount of information, which may inspire evolutionary ecologists seeking proximate mechanisms to understand thermal adaptation.

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CHAPTER 4

TEMPERATURE DEPENDENCE OF DEVELOPMENT RATE, GROWTH RATE AND SIZE: FROM BIOPHYSICS TO ADAPTATION

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Abstract

In insects, temperature strongly influences phenotype, and insects differ in sensitivity to temperature. The viable temperature range might be narrow or wide, and body size often changes with development temperature. Insect development rate depends strongly on temperature, while insect growth rate does so to a lesser extent. Development rate and growth rate show a more or less triangular shape with temperature, rising slowly and almost linearly with temperature to a maximum rate at a fairly high temperature, and decreasing steeply after at higher temperatures. Insect size in many species decreases with increasing temperature over a large part of the viable temperature range. The decrease in adult size indicates that development rate is more temperature sensitive than growth rate. The decrease might be very slight, almost amounting to temperature compensation.

Many models have been proposed to describe the temperature dependence of development rate, from the degree-day summation to a model based upon biophysics. We present the degree-day model and the biophysical Sharpe-Schoolfield model. We prefer the latter model as it has a clear biophysical base, and provides an accurate description of the temperature dependence of biological rates.

We detail the possibilities of the Sharpe-Schoolfield model. (i) It can be used to describe phenotypic plasticity in development rate, growth rate and insect size. (ii) Any change in parameters in the model immediately explains why genetic variation for phenotypic plasticity can be found. (iii) The optimal temperature for organismal functioning is part of the model. This optimal temperature proves not to be identical to the temperature of highest development rate or highest growth rate. (iv) Some of the parameters in the model can be held to describe the boundaries of the viable temperature range. The Sharpe-Schoolfield model can be used to specify in how far the boundaries of the viable temperature range and the temperature dependence of the development rate could be determined by the same biophysical parameters.

By way of the Sharpe-Schoolfield model, biophysics can be used to explain size differences over temperatures and geographical clines in adult body size. Selection on development rate or growth rate would translate into selection on the parameters of the model. So would selection for enzyme efficiency or enzyme stability. The Sharpe-Schoolfield model can therefore be used to link adaptation at the physiological level to phenotypic plasticity in body size. We can see why phenotypic plasticity is adaptive, or not, what traits are the prime movers of adaptation and what traits might be easily observed but not be adaptive themselves.

Introduction

Temperature has pervasive effects on all biological systems, and it strongly influences phenotypic plasticity. The basis of this is that all biochemical reactions are sensitive to temperature, increasing in rate with increasing temperature (Hochachka and Somero 1984). In ectothermic organisms, all biochemical reactions have to be very precisely balanced and coordinated to enable the organism to perform over a range of temperatures: very different temperature sensitivities for biochemical reactions essential for life functions would lead to a speedy surmise of an organism at any temperature change. Thus, temperature coefficients of life functions have to be balanced over a range of temperatures to ensure proper functioning of organisms when temperature varies (Hochachka 1991). In ectotherms, adaptation to ambient temperatures is a physiological, behavioral, and evolutionary task, and temperature coefficients are shaped by natural selection (Clarke 2003).

Temperature is, from pole to pole, a major determinant of life histories: of lifetime patterns of development, differentiation, growth, storage of reserves, age of maturity, fecundity patterns and longevity (Atkinson 1996, Nylin and Gotthard 1998). Life histories summarize selection patterns. Development time, fecundity, and longevity are all temperature dependent. Adaptation to any environmental temperature will, therefore, be reflected in an integrated life history pattern for that temperature (Gotthard and Nylin 1995). To understand temperature adaptation it is necessary to know how proximate mechanisms influence organism performance and fitness (Pigliucci 1996, Angilletta *et al.* 2003).

Through its influence on the individual organism's life history, temperature influences not only population density (Huey *et al.* 1999), but also community structure (Petchey *et al.* 1999). The outcome of virtually all organismal interactions, including herbivore-plant, host-parasite, and prey-predator relationships and others, depends, in part, on temperature (Harrington *et al.* 1999). The influence of temperature on density is especially of importance in damage by insects to crops. Temperature influences plant growth rate and insect development time, and, hence, the damage insects can do to crops. Successful biological pest management, therefore, requires a detailed understanding of the temperature sensitivities of the plant, insect pests, insect predators and parasitoids and subsequent selection regimes (Gilbert and Raworth 1996).

Observations on temperature dependence of viability, development rate and adult size

Viability, development time and adult size of insects and ectotherms in general are plastic, and strongly depend upon temperature. The natural viable range of temperatures allowing survival and development can differ strongly between species, and this correlates with habitat. Hence, alpine grylloblattids survive quite nicely at around 0^{o}C, but will succumb when temperatures exceed 12°C (Morrisey and Edwards 1979), whereas some desert-inhabiting insects are active at 50^{o}C, but relatively sluggish at 20^{o}C (Chapman 1982). Tropical insects with low climatic variability appear to have a narrower developmental temperature range than temperate insects living in areas with high climatic variability. This pattern has been found in amphibians (Snyder and Weathers 1975) and fish (Brett 1970). Data on temperature ranges of insects from different latitudinal ranges are, however, surprisingly difficult to come by. Within the genus *Drosophila,* for example, species differ in upper and lower temperatures that allow full development (Table 1). Temperate species seem to have shifted their viable range to a wider and lower temperature region. The tropical species seem to have a higher lower boundary temperature, but not an appreciably higher upper temperature to the viable range. As a consequence, the viable temperature range is narrower in tropical than in temperate and cosmopolitan species (Honěk 1996). What genetic basis such differences might have is

unknown. A wider thermal range of the fruitfly *Dacus tryoni* evolved in possibly less than hundred years after spreading from tropical regions to the temperate climate of southern Australia, but this wider thermal range was attributed to hybridization (Lewontin and Birch 1966).

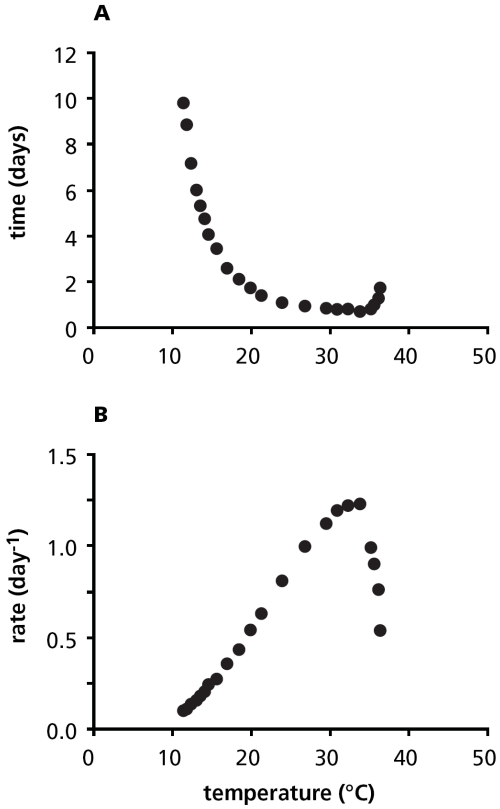

Fig. 1. Egg development as a function of constant development temperature for *Dacus cucurbitae* (Coq.). Fig. 2 from Wagner *et al.* (1991), after data from Messenger and Flitters (1958). **A**. Development time. **B**. Development rate.

Table 1. Temperature range for development in *Drosophila* species.

Species	Temperature range	Ecology
D. melanogaster	12-32[a]	cosmopolitan
D. simulans	12-32[a]	cosmopolitan
D. yakuba	13-31[a]	tropical (Africa)
D. ananassae	16-31[b]	tropical (India)
D. iri	17-32[a]	tropical (Africa)
D. frabura	16-28[a]	tropical (Africa)
D. willistoni	15-29[b]	tropical (Brazil)
D. funebris	10-29[b]	temperate (France)
D. subobscura	6-26[b]	temperate (France)

[a] (David *et al.* 1983b) [b] (Gibert and de Jong2001b)

Over the viable temperature range, a graph of development time versus temperature has almost the shape of a mirrored and very inclined capital-J. Development time increases steeply when the temperature drops. In the middle range of temperatures the reaction norm of development time changes relatively slowly but significantly with temperature. At the highest

temperatures, development time increases again. Development rate is as often quoted as development time; by definition, development rate equals 1/(development time). When development rate rather than development time is studied, relatively small differences in development time at fast development emerge as relatively large differences in development rate, and the large differences in development time near the lower border of the viable temperature range emerge as small differences. In Fig. 1, data for development rate and development time of the fly *Dacus cucurbitae* (Coq.) are plotted. The reaction norm of development rate shows the triangular shape with temperature that is very pervasive over biological rates (Huey and Kingsolver 1989). The left, increasing leg of the reaction norm rises slowly and almost linearly (Honěk and Kocourek 1988). After a maximum, the decline in development rate is precipitous, and often very short due to failure to survive. The downturn in the reaction norm and low viability are generally related and must be due to the same temperature sensitive physiology.

In ectotherms, increased developmental temperature often results in reduced adult body size (Atkinson 1996). In 91 out of 109 studies, adult ectotherm body size decreased with temperature (Atkinson 1996). It has been widely realized that the ratio of growth rate to development rate has to be altered if rearing temperature alters adult size (Atkinson 1996). Both growth rate (the rate of biomass increase), and development rate (the rate of tissue differentiation) increase with temperature. However, if development rate increases faster with increasing temperature than growth rate, adult size decreases.

To date, satisfactory explanations are lacking for this general phenomenon. Explanations are of two types: physiological and evolutionary: physiological explanations range from Von Bertalanffy's explanation (see Atkinson 1996, Atkinson and Sibly 1997) to the influence of temperature on cell size. Von Bertalanffy's explanation involves weight derived from different rates of anabolism and catabolism, where anabolism is less temperature sensitive than catabolism. Von Bertalanffy's explanation is a special case of the hypothesis of a growth constraint due to temperature. Such a constraint would result from a shortage of resources that is exacerbated by an increase in size or an increase in temperature. In an experimental study with *Drosophila melanogaster*, Frazier et al (Frazier *et al.* 2001) found that temperature and oxygen availability interacted in the determination of adult size. Hyperoxia increased adult body size at higher rearing temperatures, but at lower rearing temperatures hyperoxia had a very small effect on body size. Flies reared in hypoxic conditions were generally smaller, had longer eclosion times, slower growth rates, and reduced survival -- all signs of adverse conditions. At cooler temperatures, hypoxia had relatively modest or non-significant effects on development, while at higher temperature the effects of hypoxia were large. Whether different geographical populations of *Drosophila melanogaster*, with different body size and different temperature sensitivity, all react this way has not been established, but Frazier *et al.*'s (2001) study is the most explicit on the subject of growth constraints by temperature. Another physiological approach is the study of cell size. Cell size in invertebrates increases at lower temperature (Van Voorhies 1996, de Moed *et al.* 1997a, French *et al.* 1998, Blanckenhorn and Llaurens 2005). This appears to be a main mechanism causing larger adult size in insects; larger cell size per se remains unexplained but might be a consequence of limited change in cell number together with an increase in biomass at lower temperatures.

An evolutionary explanation of larger adult size at lower development temperature is as problematic, despite the progress claimed on the physiological problem (Berrigan and Charnov 1994). Low temperature selects for larger adult size if food and length of season are not limiting, as is the case in *Drosophila melanogaster* in laboratory studies (Partridge *et al.* 1994, Bochdanovits and de Jong 2003) In the cosmopolitan *Drosophila melanogaster* multivoltine temperate populations have larger body size than multivoltine tropical

populations, on all continents (Robinson and Partridge 2001, reviewed in de Jong and Bochdanovits 2003). Not only are adult *D. melanogaster* from temperate populations larger at any developmental temperature, but the change in size with temperature is larger too (Noach *et al.* 1996). The higher temperature sensitivity at lower temperatures is part of the life history puzzle.

Temperature therefore presents three problems for insect size: why larger at lower developmental temperature, why larger in more temperate geographic regions and why more sensitive to developmental temperature if originating from a temperate population.

Is temperature dependence of development rate adaptive or a constraint?

The fact that all biochemical rates and most development rates are highly sensitive to temperature has been interpreted as a constraint on development rates by fundamental thermodynamics. Gillooly *et al.* (2002) and Charnov and Gillooly (2003) propose that development rate vs. temperature in their mean environment follows an exponential relationship among populations and species when corrected for body size. Charnov and Gillooly (2003) extend this model to an exponential development rate with temperature within a species. However, most thermal reaction norms of insects are linear at intermediate temperatures, not exponential. Genetic variation in thermal sensitivity has repeatedly been found (Gilbert and Raworth 1996) indicating scope for selection and adaptation rather than absolute rule by thermodynamics (Clarke 2004). Rather than describing a universal exponential function, Gillooly *et al.* (2002) describe an average temperature dependence of development rate where the most interesting biology is found in the deviations around the mean.

Biologists are tempted to regard the temperature at which the maximum development rate is reached as the optimal temperature for the organisms. This might be so in some performance characters (Bennett and Huey 1990, Angilletta *et al.* 2002a, Angilletta *et al.* 2002b), but it is not inherent in the physiology of the organism and development in particular. In *Drosophila melanogaster*, maximum development rate is reached at 28-29ºC, but the maximum in the reaction norm for the number of ovarioles is realized at 22.5ºC (David *et al.* 1983, Delpuech *et al.* 1995). The latter temperature might be nearer to the optimal functioning of the organism.

Is temperature dependent adult size adaptive, or mostly temperature compensation?

Despite higher growth and development rates at higher temperatures, adult size generally decreases with increasing temperature (Atkinson 1994, 1996). The negative slope of the thermal size reaction norm might the result of approximate temperature compensation and other proximate mechanisms, or an adaptation to selection such as increased predation on juvenile stages at higher temperatures (Sibly and Atkinson 1994). Temperature compensation is the response of organism to a change in temperature such that they can maintain homeostatic physiological functions (Clarke 2003) or maintain a specific character nearly constant. An increasing number of studies suggest that similar thermal reaction norms can be realized with very different proximate mechanisms, influenced by different tradeoffs (Angilletta *et al.* 2003). These tradeoffs can occur during allocation or acquisition of resources (*e.g.*, increased predation risk during increased intensity of foraging), or can be related to the decrement in performance within one range of the thermal environment resulting from an increase in performance within another range.

We suggest that only field and laboratory experiments, including reciprocal transplantations which measure fitness of different clones or populations with different thermal reaction norms in different thermal environments, can determine the real opportunity for adaptation (Gotthard and Nylin 1995).

Questions for the chapter

In this chapter we focus on larval development to adult size, and review several models to describe the temperature dependence of development rate and growth rate. We regard adult size as a governed by the ratio of growth rate to development rate; as both rates are temperature dependent, the temperature dependence of adult size will follow. First we examine the importance of the linearity of the thermal reaction norm for development. Then we discuss the application of a biophysical model to growth rate and development rate, and therefore to adult size. We use this biophysical model of development rate to model the viability boundaries of the thermal range. We demonstrate how variation in the model parameters can be used to model genetic variation within and between populations. A choice of the parameter values exemplifies geographical and between-species patterns in rates and size.

Models of Development Rate

Many mathematical models have been used to describe development rate as a function of temperature. For surveys of the performance of these models, see Wagner *et al.* (1991) and Kontodimas *et al.* (2004). Many of these models are empirical and statistical: they aim at an accurate description of possible data on insect development rate, without attempting to explain development rate in terms of biological mechanisms. Deductive and explanatory models however seek to understand the causes of particular development rates from physiological mechanisms that might themselves be understood in terms of lower level processes.

Of all existing descriptive models we will mention only one, the degree-day model. The degree-day model is the simplest model for development rate, over the more or less linear middle part of the temperature range where development rate increases (Honěk 1996, Honěk and Kocourek 1990). However, the model potentially has an interesting biological background, in terms of physiological time. And even more interesting, the degree-day model might relate with the molecular genetics of cell growth and cell proliferation, through the link provided by physiological time.

In biology, mechanistic explanations are usually given in terms of lower level biological, cellular, and/or biochemical processes. However, in the case of temperature dependent development rate, the lower level explanation of biological rate is in terms of a biophysical explanatory model, not a biochemical model. One model dominates this field of explanatory models based on biophysical reaction rates: the Sharpe-Schoolfield model. This model, developed by Sharpe and DeMichele (1977) and Schoolfield et al (1981), provides an accurate description of biological rates based upon plausible biophysics. Not only does the Sharpe-Schoolfield model accurately describe temperature-dependent biological rates (Kontodimas *et al.* 2004), it provides a framework of fruitful thinking on the causation of such rates.

The Sharpe-Schoolfield model closely mimics empirical data, yielding a fairly linear increase in development rate over the middle part of the viable temperature range, as does the degree-day model. The parameters of the Sharpe-Schoolfield model have to conform to constraints for this to be true. We will show how these two models can be made to connect.

Degree-day Model

Empirical observations on ectothermic plants and animals show that development can often be accurately characterized by a 'temperature sum.' This was realized as early as 1735: de Réaumur (1735), as cited by Wang (1960). The rather surprising observation was that plants or insects needed a total amount of heat to reach any particular state. This amount of heat is constant over a large part of the viable temperature range – in particular, over the mid-region where development rate increases. The actual temperature of development does not matter. The threshold temperature for an insect to develop might, for instance, be 12ºC. If development takes 20 days at 17ºC, the temperature sum equals (17- 12) × 20 = 100 degree-days. In the same species, development at 22ºC would take about 10 days, and imply an identical temperature sum of (22 - 12) × 10 = 100 degree-days. A constant temperature sum, that is, a constant number of degree-days, leads to the prediction that at 27ºC development would take 100/(27 - 12) = 6.66 days. Such simple predictions are widely used in applied entomology.

Empirical data

Constancy of the number of degree-days needed to complete development, whatever the actual development rate and temperature, depends upon a very simple feature of development rate. Development rate increases with temperature over the middle region of the viable temp increases in a linear manner, to a very good approximation (Figs. 2 & 3). A linear regression line can be fitted through this part of the curve. Where the regression line representing development rate crosses the temperature axis (the x axis) is the threshold point *h* for the development rate. It is taken to indicate a zero growth rate, but is in reality, a temperature that is slightly above the biological temperature of zero growth, but quite sufficiently near it for practical purposes. The equation for development rate r_d over its middle part can be written as: $r_d = c\,(T-h) = c\,x$, where T is temperature in ºC, $x = T-h$ is the difference between the actual temperature and the threshold temperature, and c is the slope of the linear regression line representing development rate. Development time equals $t_d = 1/r_d = 1/cx$. Therefore, the total number of degree-days $t_d x$ equals $1/c$. As long as the development rate is a linear function of temperature, the number of degree-days will be constant, and equal to the inverse of the slope of development rate with temperature. Once we know that development rate is a linear function of temperature, this constant temperature sum based on the temperature (above threshold) and development time, is a mathematical certainty.

In the literature, many degree-day values can be found, as the method is actively used in predicting development in insect pests (for instance, Honěk and Kocourek 1988, 1990). In the journal *Environmental Ecology* many articles reporting on insect development mention degree-day values or average degree-day values. For instance, Judd *et al.* (Judd *et al.* 1991) give both an average degree-day value and a standard error (602 ± 13 degree days) in their study of development in the pepper maggot. Empirical degree-day values are not absolutely constant, even if the feeding environment and all other environmental influences are the same. Environmental differences other than temperature, such as food, population density, and allospecifics, influence the number of degree-days, and, in addition, development rates sometimes deviate slightly from linearity. However, the number of degree-days to reach maturity is constant enough to be of biological interest. It implies that the linearity of development rate as a function of temperature is more than a statistical first approximation: it seems a biological property. Therefore, it is a biological question how linearity of development rate is caused.

Physiological time

Van Straalen (1983) noted that the concept of degree-days or of a temperature sum could be regarded as a transformation from physical time to physiological time. Physiological time is very useful in biology, as it can be used to construct models that are time-invariant. A physiological time-scale can be defined as follows (van Straalen 1983):

> A physiological time-scale for a specified biological process is a time-scale obtained by transforming a physical time-scale so that the rate of the process becomes time-invariant in physiological time.

The relationship between physiological time, physical time, and temperature, is very important for understanding animal development, ultimate phenotype, and constraints. If different traits possess the same physiological time scale under temperature change, the relation between those traits remains the same for all temperatures. Two traits that have the same physiological time over temperature change are temperature-invariant relative to each other; hence, their ratio remains constant at all temperatures. Obviously, the great multitude of biochemical/physiological processes that underlie an organism's development must possess a similar temperature-specificity, or be relatively temperature-invariant, in order to produce individuals that function properly when exposed to different temperatures. However, few traits, especially physiological traits, are truly temperature invariant; circadian rhythm might be one of the few temperature invariant traits. Temperature invariance might be difficult to achieve for physiologically dependent traits, and most traits are ultimately physiologically dependent.

The argument of van Straalen (1983) demonstrates the relation between the linear portion of the development rate, the constant temperature sum and physiological time. Van Straalen's mathematical argument is presented in verbal form in Box 1. The argument concerns when a transformation between physical time and physiological time can be defined. A linear development rate implies a constant temperature sum for total development. A constant temperature sum implies similar development under a physiological time scale. Development is time-invariant under a physiological time scale under the condition of a constant temperature sum, i.e., of a development rate that is linear with temperature.

Biology of physiological time

The scaling of the linear development rate to constant physiological time is biologically interesting. What might correspond, in organismal time, to the physiological time implied by the temperature sum? A possible answer to this question is number of cell divisions in the developing organism.

This possible answer derives from detailed investigations into the components of morphological phenotypic plasticity in insects, in particular *Drosophila melanogaster*. This species shows the classical tilted triangular shape of development rate as a function of temperature (Fig. 2). *D. melanogaster* adult body size has been extensively investigated for phenotypic plasticity. The most phenotypically plastic trait is wing size; thorax size might be more generally indicative of body size and has lower but still appreciable phenotypic plasticity (Noach *et al.* 1996, Karan *et al.* 1998, Karan *et al.* 1999). The *Drosophila* wing

BOX 1. Linear development rate and physiological time

We explore when a conversion from varying physical time to constant physiological time might be possible, assuming that insect development time depends upon temperature. The development rate *r* from egg to adult depends upon temperature, but is constant, at a specific temperature, over the developmental period.

The question is whether it is possible to define a physiological time scale such that the rate of the developmental process is constant over physiological time and independent of temperature. This temperature-independent physiological rate would be given by *k* if it exists; 1/*k* therefore has the unit time, and is the unit in which physiological time is measured.

The starting point of the conversion between physical time and physiological time is that the same amount of development is taking place at both time scales (as it is the same developing organism). This amount of development can be visualized as a length of time times the development rate over that time. Total development over a length of physiological time $\Delta\tau$ times the physiological development rate *k* equals $k\Delta\tau$. Total development over an equivalent length of physical time Δt times the physical development rate *r* equals $r\Delta t$. Since both expressions concern the same development, $k\Delta\tau = r\Delta t$, and the potential conversion of physical time to physiological time can be given as $\Delta\tau = r\Delta t/k$. Here r and Δt both depend upon temperature, but the temperature dependence should cancel in the conversion if a temperature independent rate *k* is possible in physiological time.

The amount of development has to be proportional to the temperature sum in physical time. This is the essence of temperature dependence. Total developmental time t_a from egg to adult equals 1/*r* in physical time. The temperature sum of completed development therefore equals $t_a(T-h) = \frac{1}{r}(T-h)$, where *h* ºC equals the minimum temperature for development. For a conversion from physical time to constant physiological time to be possible, the temperature sum $\frac{1}{r}(T-h)$ should be independent of temperature for all temperature dependent rates *r*.

The temperature sum over total development is constant if the temperature dependent rate equals $r = c(T-h)$. In that case, the temperature sum equals $1/c$ and is constant. A constant temperature sum implies that development is independent of temperature. Therefore rt_a is constant, independent of temperature, as is each $r\Delta t$, and as $k\Delta\tau = r\Delta t$, a constant physiological time exists. Over total development, τ_a is defined to equal 1. Physiological time is measured as 1/*k*. Development is time-invariant under a physiological time scale.

The linearity of development rate with temperature implies a scaling of development rate to constant physiological time; only a linear development rate has this property (van Straalen 1983).

Growth rate and development rate might both be linear with temperature. If so, both growth rate and development rate can be scaled to a temperature-independent physiological time scale, if not directly to the same time scale. However, different temperature-independent physiological time scales $1/k_g$ and $1/k_d$ are related to each other by a simple conversion factor. The temperature sums for growth rate and

development rate must be constant, as otherwise no physiological time scales can be defined. If so, the division of the temperature sums is temperature independent and equal to c_d / c_g. This constant gives a conversion factor between time scales.

Body size, as the division of growth rate by temperature rate, will be constant if the minimum h ºC is identical for the two rates. This will be clear from the expression for size, as size is found as size $= \frac{c_g (T - h_g)}{c_d (T - h_d)}$. At equal minimum temperature, size = c_g / c_d. Temperature independent body size therefore implies a direct relation of the physiological time scales of the two rates.

The relative duration of stages or instars are often found to be independent of temperature (Jarošík *et al.* 2002). If development rate is linear with temperature for each instar *i* according to $r_i = c_i (T - h_i)$, a constant temperature sum is present for each instar, and a separate physiological time scale can be defined for each instar. The division of the temperature independent temperature sums for two instars *i* and *j* is temperature-independent and equal to c_i / c_j, and the relative duration of the instars is constant over temperature. If the instars have identical minimal temperatures *h*, the relative duration of the instars equals the division of their development rates, c_i / c_j.

lends itself easily to the determination of cell size and cell number. Geographic variation in wing size is mostly but not exclusively determined by changes in cell number, cell number being higher in flies from temperate latitudes (De Moed *et al.* 1997b, Zwaan *et al.* 2000). Larger wing size, due to lower rearing temperature, proved to be mainly determined by larger cell size, with little or no effect of cell number (*e.g.*, De Moed *et al.* 1997ab, French *et al.* 1998). This effect of cell size might be wing-specific or population-specific. Therefore, Azevedo and coworkers (2002) surveyed phenotypic plasticity in cell size of the wing, the basitarsus of the leg and the cornea of the eye of *Drosophila melanogaster* from two populations at opposite ends of the South American latitudinal cline in body size. They found that lower rearing temperature increases wing size, leg length and eye size, through an effect on epidermal cell size, but without a significant change in cell number.

The explanation of the environmental effect on body size through cell size might be found in the growth characteristics of the imaginal discs. In *Drosophila*, the epidermis of the adult head and of the adult thoracic segments is formed by separate imaginal discs that grow and differentiate inside the developing larva. Given full larval nutrition and constant temperature, the cell proliferation of these imaginal discs might be intrinsic: that is, they might grow to a predetermined number of cells, at least for a specific *Drosophila* strain (Bryant and Simpson 1984, Bryant and Levinson 1985). Cell size can therefore be regulated and potentially selected apart from cell number. Cell size seems mainly determined by growth temperature. Constant cell number in imaginal discs (within a line) seems independent of temperature. Therefore, cell number makes a good candidate as deriving from organismal physiological time during development. In other words, the same number of cell divisions might be needed to complete animal development, independent of temperature, at least in the mid-region of the viable temperature range. This suggests that all biochemical processes of cell division have rates that increase linearly with temperature over this range. Of course, the rates of all chemical and biochemical reactions are inherently temperature sensitive (Hochachka and Somero 1984). This brings us to the other type of model of development rate, a deductive and explanatory model based upon the biophysics of reaction rates.

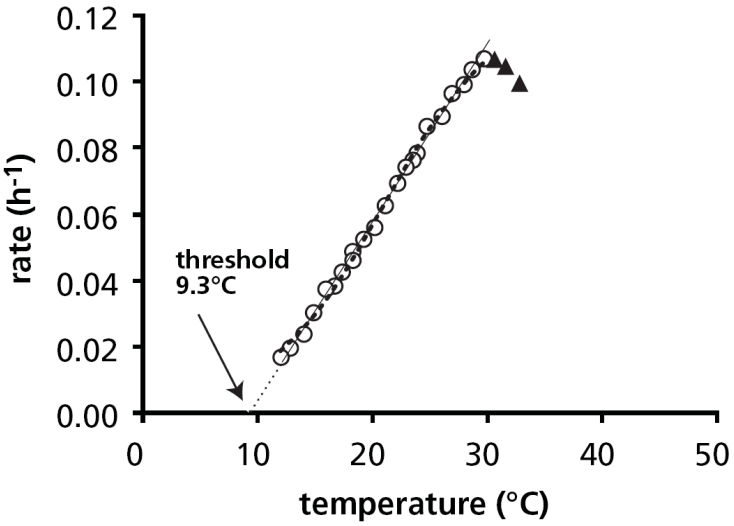

Fig. 2. Development rate of prepupae of D*rosophila melanogaster*. Fig. 1 from Gilbert and Raworth (1996), using data from Bliss (1926).

Sharpe-Schoolfield Model

The Sharpe-Schoolfield model is based upon two principles: (i) temperature dependent reaction kinetics per active enzyme molecule; (ii) reversible inactivation of enzyme molecules at high or low temperature. Both the reaction kinetics and the inactivation of molecules use classical models of biophysical reaction rates. We will use these models without more explanation of biophysical detail than is necessary for understanding the biology. The simplest version of the theory of biochemical reactions can be found in Willmer *et al.* (2000). Slightly more advanced treatments can be found in the discussion of biochemical adaptation by Hochachka and Somero (1984) or in Lowry and Richardson (1987). Watt (1968) gives a clear ecological introduction for practical biologists.

Model based upon biophysics

Temperature dependence of reaction rates has traditionally been described by an empirical equation due to Arrhenius (around 1900) and a more theoretical equation due to Eyring (1935). Sharpe and DeMichele (1977) developed a model for development rate based upon the Eyring equation. The parameters of this model conformed to the usage in the biophysics of chemical reactions, and had no direct biological interpretation. The model made a number of assumptions.

The first assumption is that development is regulated by a single control-enzyme whose reaction rate determines the development rate of the organism. This assumption might be less restrictive than it seems at first look. Sharpe and DeMichele (1977) show how several limiting enzymes, for parts of the temperature range, leave the overall impression of determination of the development rate by one controlling enzyme over all the range. Hence, although the physiological reality might be several enzymes, the model can proceed as if one enzyme is in control of development over all the range. Moreover, development rate is proportional to the product of the concentration of active molecules of the control enzyme and their temperature-dependent reaction rate. For each enzyme molecule in the active state, the reaction rate exponentially increases with temperature.

The second assumption is that the control-enzyme can exist in two temperature-dependent reversible inactivation states as well as in an active state; one reversible inactivation state pertains to high temperature, the other to low temperature.

Schoolfield *et al.* (1981) modified the model of Sharpe and DeMichele to make it more convenient for biological interpretation. They introduced a reference reaction rate that absorbed physical constants and the entropy of activation, combined other parameters and succeeded in writing the Sharpe and DeMichele model in a way that can be easily understood and now has parameters that all have clear biological relevance.

The classical biophysical Eyring equation describes a reaction rate's exponential increase with temperature. The Eyring equation describes temperature sensitivity of reaction rates without enzyme inactivation. Schoolfield et al (1981) give the Eyring equation in the original parameters (their Equation 2). In the parameters, as defined by Schoolfield *et al.* (1981), the Eyring equation reads:

$$r(T) = \rho \frac{T}{T_{ref}} \exp\left[\frac{H_A}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right] \qquad \text{Equation 1}$$

The ratio $r(T+10)/r(T)$ is known as Q_{10}, a widely used rough and ready guide to the temperature sensitivity of reactions.

In the Eyring equation, reaction rate $r(T)$ as a function of temperature T is given as a modification of a reference reaction rate ρ at a reference temperature T_{ref} (in °K). The dependence of the reaction rate on temperature is given by the temperature sensitivity coefficient H_A (in J mol^{-1}), officially the enthalpy of activation of any reaction that is catalyzed by a rate-controlling enzyme (Hochachka and Somero 1984, pg 379-380, notation simplified); R is the universal gas constant (8.314 J $K^{-1} mol^{-1}$). The exponential factor in Equation 1 yields a dimensionless scaling to temperature of the reference reaction rate ρ. The exponential factor is larger than 1 if temperature is higher than the reference temperature and the reaction rate is then higher than the reference reaction rate. Conversely, the exponential factor is less than 1 if the environmental temperature is lower than the reference temperature, and the reaction rate is then lower than the reference reaction rate.

What rate the equation refers to is specified by the units of the reference rate ρ. If the biological rate described by Equation 1 refers to development rate, ρ has the unit $time^{-1}$. If the considered rate refers to growth rate, then ρ's units are biomass per time. The same equation applies to any intended temperature sensitive rates that are determined by biochemical reactions.

Biological rates show this exponential increase at most across a very limited temperature range. At middle temperatures, rates are almost linear. At higher temperatures, development rate usually sharply decreases. At low temperatures, development rate slowly approaches zero. Any feasible model designed to explain all of development rate has to include the high and low temperature behavior of biological rates, and accommodate the linearity. A very useful assumption is that the sharp downturn in development rate at high temperature is due to enzyme inactivation. This is a particularly plausible assumption as the downturn in development rate often is near the high end of the viable range for development (Fig. 3).

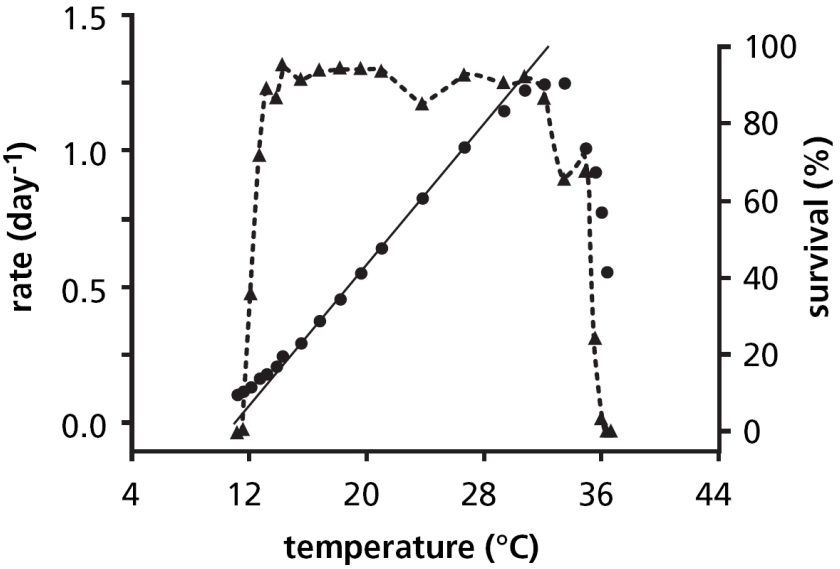

Fig. 3. Development rate (circles) and survival (triangles) for eggs from *Dacus cucurbitae* (Coq.). Fig. 4 from Wagner *et al.* (1991), after data from Messenger and Flitters (1958).

Sharpe and De Michele (1977) therefore proposed to modify the Eyring equation in order to describe biological rates better. The Eyring equation can be modified by the probability that any enzyme molecule is active at a given temperature. Any enzyme molecule is supposed to be subject to reversible inactivation at both high and low temperature; the reversible inactivation processes or sensitivities at the two temperature extremes are supposed to be independent. The probability for the enzyme to be active can be thought of as proportional enzyme efficiency over temperatures. This enzyme efficiency is characterized by two parameters at low temperature and by two parameters at high temperature. At low temperature, the parameters are: (1) the temperature at which 50% of all molecules of a rate-limiting protein or enzyme are reversibly inactive due to cold, the lower boundary temperature T_L, in °K; and (2) the specific sensitivity to cold inactivation, the cold inactivation coefficient H_L. The sensitivity to heat inactivation at low temperature is formally the change in enthalpy associated with low temperature inactivation of the enzyme, and expressed in J mol^{-1}. At high temperature, the parameters are: (1) the temperature at which 50% of all molecules of a rate-limiting protein or enzyme are reversibly inactive due to heat, the upper boundary temperature T_H, in °K; and (2) the specific sensitivity to heat inactivation, the heat inactivation coefficient H_H. The sensitivity to heat inactivation at high temperature is formally the change in enthalpy associated with high temperature inactivation of the enzyme, and again expressed in J mol^{-1}.

The enzyme efficiency, as probability P_T for the enzyme to be active as a function of temperature, is given by:

$$1/P_T = 1 + \exp\left[\frac{H_L}{R}\left(\frac{1}{T_L} - \frac{1}{T}\right)\right] + \exp\left[\frac{H_H}{R}\left(\frac{1}{T_H} - \frac{1}{T}\right)\right] \qquad \text{Equation 2}$$

Incorporation of the probability of the enzyme to be active yields the Sharpe-Schoolfield-equation for any biological rate as a function of temperature:

$$r(T) = \rho \frac{TP_T}{T_{ref}} \exp\left[\frac{H_A}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right] \qquad \text{Equation 3}$$

The six parameters of the Sharpe-Schoolfield-equation can be used in paired combinations.

The reference rate ρ and the temperature sensitivity coefficient H_A refer to the total temperature range. These two parameters by themselves might be sufficient to describe a short middle region of development rate, in a two-parameter version of the Sharpe-Schoolfield model (Fig. 4a). Two parameters, T_L and H_L, are specific for the low-temperature range, and two parameters, T_H and H_H are specific for the high temperature range. Two versions of a four-parameter model exist, one at low temperatures with reference rate ρ and temperature sensitivity coefficient H_A together with lower boundary temperature T_L and cold inactivation coefficient H_L (Fig. 4b), and one with parameters ρ and H_A together with upper boundary temperature T_H and heat inactivation coefficient H_H, at high temperatures (Fig. 4c). Over the total temperature range, the full six-parameter model can be used (Fig. 4d). These four figures are given by Wagner *et al.* (1991) as examples of the four sub-models and their good fit to data on insect development.

Parameter variation

The Sharpe-Schoolfield-equation possesses in total six parameters, two from the Eyring equation and four from the description of temperature dependent reversible enzyme activation. Together, these parameters describe development rates that are overall similar in shape but might be very different in detail.

Different shapes of the probability P_T for the control enzyme to be active represent biologically different cases of enzyme adaptation to temperature. The probability of the rate-determining enzyme to be active as a function of temperature decreases at both high and low temperature; the probability might reach a value of 1, but this is not necessary. High absolute values of the inactivation coefficients H_L and H_H at the lower and upper boundary temperatures T_L and T_H imply a wider plateau and a faster decrease of the probability that the rate-determining enzyme is active. In Fig. 5a, two enzymes with different parameters are shown as examples. One enzyme has parameters H_L and H_H that are large in absolute value; therefore the enzyme activity declines steeply both at high and low temperature. The other enzyme has very low values for the parameters H_L and H_H , and, as a consequence, the probability to be active increases and decreases slowly with temperature. In this case, the enzyme need not be fully active at any temperature. In Fig. 5b, two enzymes are shown that are a high and low temperature specialist, due to one temperature inactivation that goes very rapid and another temperature inactivation that is very gradual. The maximum in the probability P_T for the control enzyme to be active is at high or low temperature. This contrast provides a possibility to model enzyme specialization to high or low temperature (Hochachka and Somero1984).

The Eyring equation describes an exponential increase of a biological rate with temperature; higher temperature sensitivity coefficient H_A implies a faster increase at temperatures higher than the reference temperature (Fig. 5c) and higher reference rate ρ implies both a higher rate at the reference temperature and a faster increase with temperature

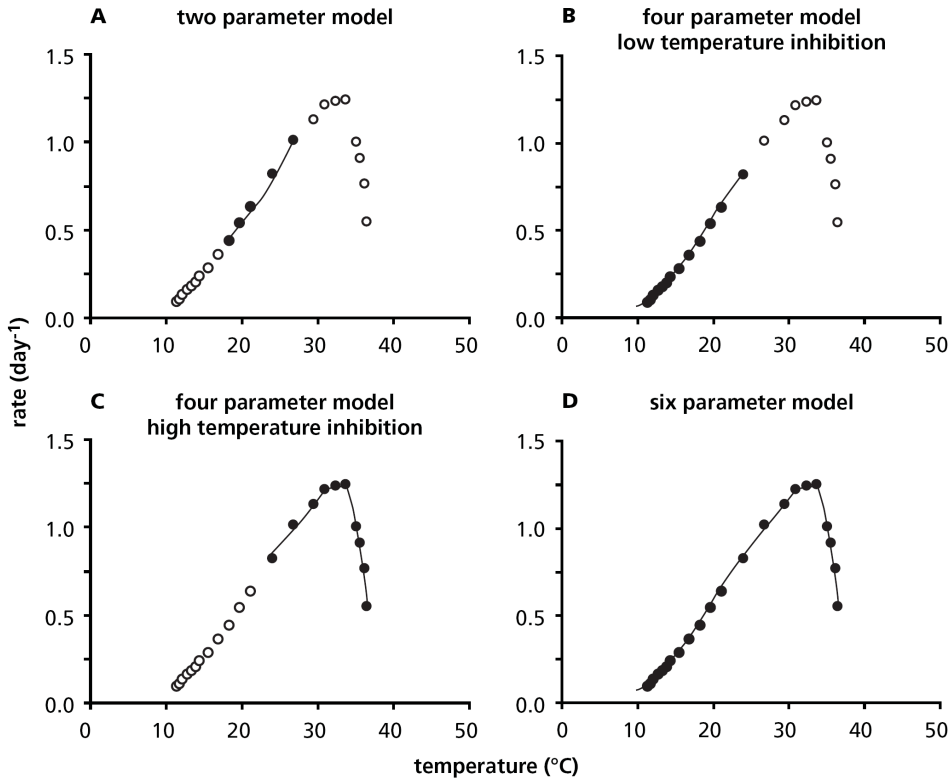

Fig. 4. Sharpe-Schoolfield model fitted to the data from Messenger and Flitters (1958). **A**. Two parameter model over the middle temperature range using ρ and H_A. **B**. Four parameter model over the low temperature using ρ and H_A, as well as H_L and T_L. **C**. Four parameter model over the high temperature using ρ and H_A, as well as H_H and T_H. **D**. Six parameter model for the entire data set, ρ and H_A, as well as H_L and T_L, as H_H and T_H . Fig. 5 from Wagner et al (1991).

(Fig. 5d). Changing parameter combinations lead to different development rates. The steepness of the rates depends highly on the inactivation parameters H_L and H_H (Figs. 5e,f). The maximum of the rates is for a large part dependent upon H_A and ρ, larger H_A implying higher development rate (Figs. 5e,f).

Arrhenius plot and parameter estimation

The six parameters of the Sharpe-Schoolfield model have to be estimated in order to investigate the validity and sufficiency of the model in describing empirical data on development rate. The high temperature range of development rate often shows a sudden down-curve. Likewise, the low temperature range of development might show a development rate that approaches zero asymptotically. Many data points are necessary to get accurate estimates in both of these regions. However, data collection at low temperatures represents a disproportionate investment in effort compared to high temperatures, because of the greater time to complete development, and the problems associated with keeping insects healthy at low temperatures.

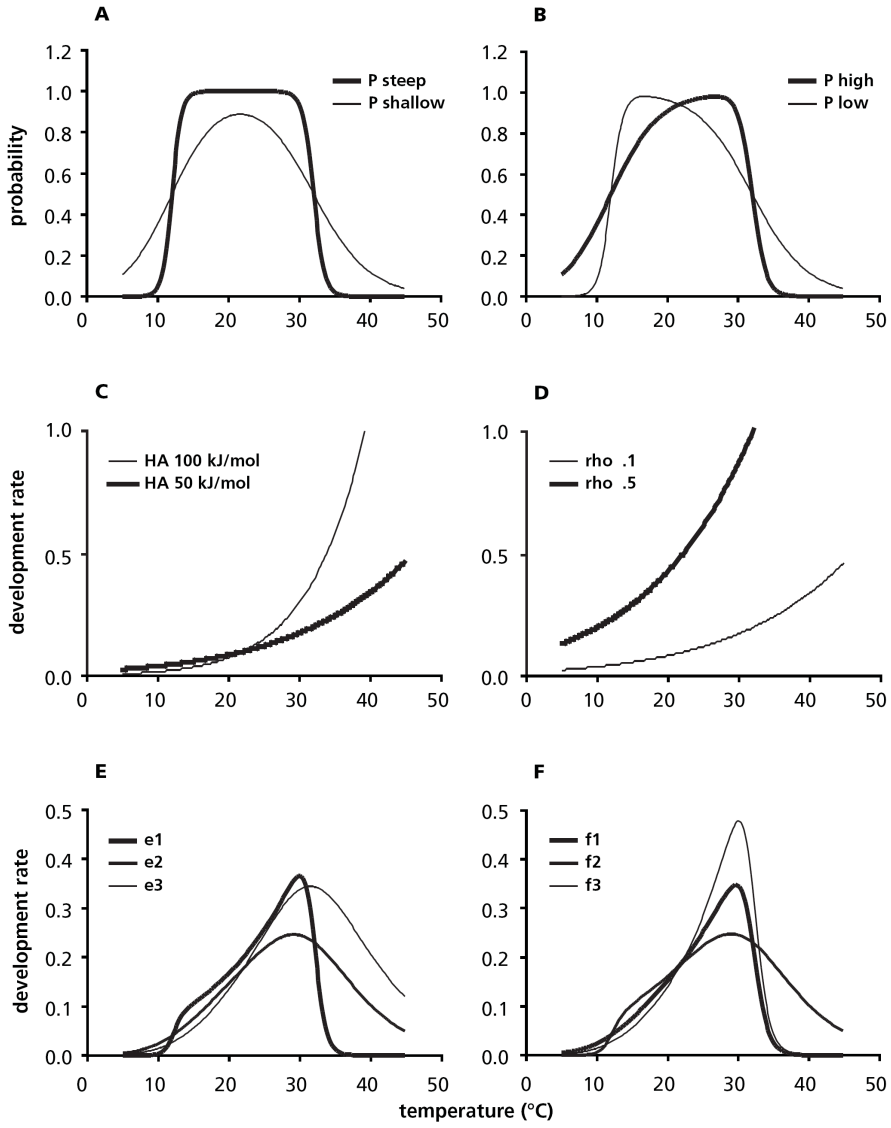

Fig. 5. Influence of parameter variation in the Sharpe-Schoolfield model on development rate. In all cases, T_{ref} = 295ºK = 2 1.8ºC, T_H = 305ºK = 3 1.8ºC, and T_L = 285ºK = 1 1.8ºC. **A**. Parameter variation in denominator *P*: H_H = 1000 kJ mol^{-1} , H_L= - 1000 kJ mol^{-1} (steep) versus H_H = 200 kJ mol^{-1}, H_L= -200 kJ mol^{-1} (shallow). **B**. Parameter variation in denominator *P*: H_H= 800 kJ mol^{-1} and H_L= -200 kJ mol^{-1} (skewed to high temperature) versus H_H= 200 kJ mol^{-1} and H_L= -800 kJ mol^{-1} (skewed to low temperature). **C**. Variation in H_A: 100 kJ mol^{-1} and 50 kJ mol^{-1}: note identity at reference temperature at ρ=0.1. **D**. Variation in ρ: ρ = 0. 1 t^{-1}, ρ = 0.5 t^{-1} at H_A=50 kJ mol^{-1}. **E**. Variation in rate: (e1) H_H = 1000 kJ mol^{-1}, H_L= - 1000 kJ mol^{-1} with H_A= 60 kJ mol^{-1} and ρ =0.2 ; (e2) H_H = 200 kJ mol^{-1}, H_L= -200 kJ mol^{-1} with H_A= 60 kJ mol^{-1}and ρ =0.2; (e3) H_H = 200 kJ mol^{-1}, H_L= -200 kJ mol^{-1} with H_A= 90 kJ mol^{-1}and ρ =0.2. **F**. Variation in rate: (f1) H_H = 800 kJ mol^{-1}, H_L= -200 kJ mol^{-1} with H_A= 60 kJ mol^{-1}and ρ =0.2 t^{-1} ; (f2) H_H = 200 kJ mol^{-1}, H_L= -800 kJ mol^{-1} with H_A= 60 kJ mol^{-1} and ρ=0.2 t^{-1}; (f3) H_H = 800 kJ mol^{-1}, H_L= -200 kJ mol^{-1} with H_A= 90 kJ mol^{-1} and ρ =0.2 t^{-1}.

For statistical reasons, seven data points are minimally necessary to estimate the six parameters of the Sharp-Schoolfield model. However, it should be remembered that while seven points will estimate a curve with six parameters, the reliability of the estimates depends on the density of points along the low- and high-temperature inflections. In statistical programs like SAS or SPSS, a general non-linear regression curve-fitting module using the Marquardt Method is present and can be used. Such programs need initial parameter values. If only seven points are available, initial parameter values have to be found in a range of values published in the literature. If the number of data points is optimized for curve fitting rather than the minimum needed for statistics, an Arrhenius plot might help to estimate initial parameter values (Schoolfield *et al.* 1981, Wagner *et al.* 1984).

In an Arrhenius plot, the logarithm of development rate is plotted against the inverse of temperature, in °K. In order to make clear how estimation proceeds, we will not start with data but with known theoretical values for the six parameters of the Sharpe-Schoolfield model (Schoolfield *et al.* 1981). In Fig. 6, theoretical development rates are plotted both as rate versus temperature (Fig. 6a) and as the logarithm of the rate versus the inverse of the temperature (Figs. 6c,d). Moreover the probability for the enzyme to be active is plotted (Fig. 6b). Note that the probability for the enzyme to be active is higher than 0.9 between 16°C and 28°C. The logarithm of the development rate plotted against the inverse of temperature $1/T$ shows a more or less linear middle part, and curves down at the left (high temperature) and right (low temperature). A linear middle part corresponds to temperatures where the probability for the rate-controlling enzyme to be active equals 100%. Only then will we find linearity in an Arrhenius plot. In such a middle part of high enzyme activity, development rate can be sufficiently described by the Eyring equation. High probabilities for the enzyme to be active, greater than 90% or so, lead to an approximately linear part in the Arrhenius plot. This linear part can be used to estimate the reference rate ρ and the temperature sensitivity coefficient H_A, as the slope of the straight line equals $-\frac{H_A}{R}$ and the intercept $\ln\rho + \frac{H_A}{R}\frac{1}{T_{ref}}$.

Model fit to empirical pattern

The Sharpe-Schoolfield model is excellently suited to describe biological rates (Kontomidas *et al.* 2004). Of course, any model with 6 parameters might be expected to perform well, but the Sharpe-Schoolfield model is better able than most alternatives to describe the fine detail of development rate, especially the sudden decrease at high temperature. The initial curve fittings by Sharpe and DeMichele (1977) showed the suitability of the model. Wagner and co-authors (1984) applied the model successfully to data from a range of species, and compared the performance of the Sharpe-Schoolfield model with a range of alternative models common to entomology (Wagner et al 1991).

For *Drosophila*, some data are available for development rate (Table 2) and growth rate (Table 3). Van der Have and de Jong (1996) estimated the 6 parameters for *Drosophila melanogaster* development rate and growth rate on the base of data from David and Clavel (1967). Both rates are fairly linear with temperature over the middle range (Fig. 7a). In the developmental rate case, the rate-controlling enzyme is at no temperature more than 90% active, for growth rate the maximum probability for the enzyme to be active reaches 0.975 (Fig. 7b). The middle part of the Arrhenius plot is not really linear: as the probability for the rate-controlling enzyme to be active has no plateau at 100%, the line of the Eyring equation corresponding to the numerator only approaches the curve for the Sharpe-Schoolfield model (Fig. 7c). As the rates are fairly linear with the temperature range, the number of degree-days

BOX 2. Parameter estimation from an Arrhenius plot

Parameter estimation from an Arrhenius plot is detailed in in Schoolfield *et al.* (1981) and Wagner *et al.* (1984). Taking logarithms, the Eyring equation (Equation 1) becomes

$$\ln r(T) = \ln\rho + \ln\frac{T}{T_{ref}} + \frac{H_A}{R}\frac{1}{T_{ref}} - \frac{H_A}{R}\frac{1}{T}. \qquad \text{Equation 4}$$

Over physiological ranges from some 10°C to some 30°C, the term $\ln\frac{T}{T_{ref}}$ will be very small and can be neglected. In a plot of $1/T$ on the x-axis and ln r on the y-axis, the slope of the straight line will be then $-\frac{H_A}{R}$, and the intercept $\ln\rho + \frac{H_A}{R}\frac{1}{T_{ref}}$. The slope in an Arrhenius plot can therefore be used to estimate H_A. After estimation of H_A, the intercept can be used to estimate ρ. In Fig. 6c, the curve represents Sharpe-Schoolfield model, and the straight line its numerator, the Eyring equation.

Estimation of H_H proceeds by dividing the Eyring equation by the term from the probability of enzyme inactivation that is specific for high temperature temperature (Schoolfield *et al.* 1981, Wagner *et al.* 1984) and taking logarithms:

$$\frac{\rho\frac{T}{T_{ref}}\exp\left[\frac{H_A}{R}\left(\frac{1}{T_{ref}}-\frac{1}{T}\right)\right]}{\exp\left[\frac{H_H}{R}\left(\frac{1}{T_H}-\frac{1}{T}\right)\right]} = \rho\frac{T}{T_{ref}}\exp\left[\frac{H_A}{R}\left(\frac{1}{T_{ref}}-\frac{1}{T}\right)-\frac{H_H}{R}\left(\frac{1}{T_H}-\frac{1}{T}\right)\right]$$

$$\ln\rho + \ln\frac{T}{T_{ref}} + \left(\frac{H_A}{R}\frac{1}{T_{ref}} - \frac{H_H}{R}\frac{1}{T_H}\right) - \left(\frac{H_A}{R}-\frac{H_H}{R}\right)\frac{1}{T} \qquad \text{Equation 5}$$

The result is again linear if the logarithm of this division is plotted against $1/T$, now with slope $-(H_A - H_H)/R$ (Fig. 6d, line High). Similarly, estimation of H_L proceeds by dividing the Eyring equation by the term from the probability of enzyme inactivation that is specific for low temperature. The resulting is linear in a plot of the logarithm of this division against $1/T$ (Fig. 6d), now with slope $-(H_A - H_L)/R$. In both the high and low temperature cases, accuracy of estimation would require many data points near the temperature extremes for viability.

Estimation of T_H and T_L can proceed in two ways. On the one hand, the property that the probability for the enzyme to be active is exactly one half at both temperatures can be used. This implies that the numerator from the Sharpe-Schoolfield model can be divided by 2 to obtain a straight line that crosses the curve of the full Sharpe-Schoolfield model at T_H and T_L (Fig. 6d; (Schoolfield *et al.* 1981, Wagner *et al.* 1984)). On the other hand, in the logarithmic plot the straight line High crosses the straight line Eyring at T_H, and the straight line Low crosses the straight line Eyring at T_L (compare Equations 4 and 5; Fig. 6d).

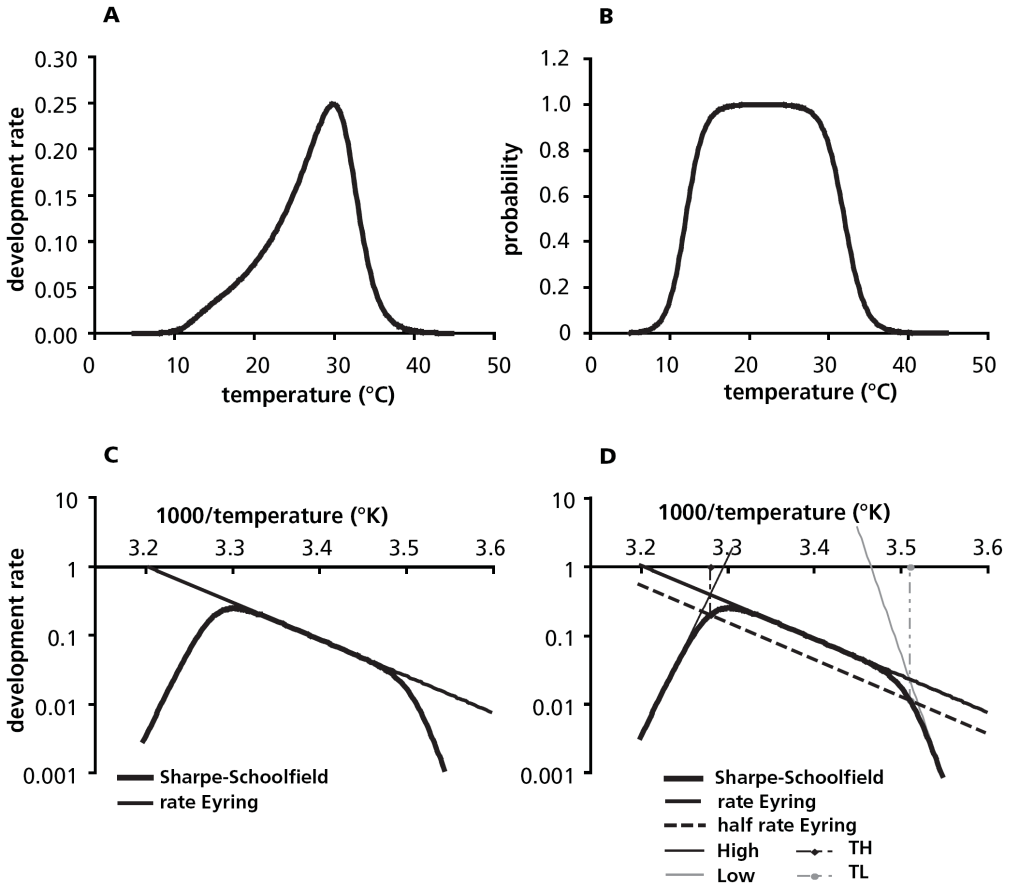

Fig. 6. Graphical estimation of parameter values. The graphs are drawn according to the parameter values H_A= 100 kJ mol^{-1}, ρ =0. 1 t^{-1}, H_H = 600 kJ mol^{-1}, H_L= -600 kJ mol^{-1}, T_{ref} = 295ºK = 2 1.8ºC, T_H = 305ºK = 3 1.8ºC, and T_L = 285ºK = 1 1.8ºC. Figs. 6a and 6b give the data. Figs. 6c and 6d the estimation. **A**. Rate. **B**. Probability enzyme active. **C**. Ln rate versus 1/T : estimation of H_A and ρ from the 17ºC to 26ºC temperature range as H_A = 102.46 kJ mol^{-1} and ρ = 0.099 t^{-1}. **D**. Ln rate versus 1/T: estimation of H_H, H_L, and T_H, T_L as H_H = 599.9 kJ mol^{-1}, H_L = - 599.9 kJ mol^{-1}, and T_H = *305*ºK, T_H= 285ºK.

can be estimated. Linear regression on temperature for the temperature range 11ºC to 30ºC yields a threshold of 10.2ºC for development rate, and 9.6ºC for growth rate. The actual number of degree-days is computed as the product of the development time at a specific temperature as found from the Sharpe-Schoolfield model, times the degrees over the threshold. The number of degree-days is not constant (as the development rate is not perfectly linear) but is restrained to a fairly narrow region over the feasible range of development temperatures (Fig. 7d).

Estimates of all six parameters are available for a further two *D. melanogaster* populations and two *D. simulans* populations, from the same temperate (Houten, The Netherlands) and Mediterranean (Adana, Turkey) locations. Over the populations, no species differences in development rate or growth rate were found (Figs. 8a,b). The probabilities for the enzyme to be active never reached 100% (Figs. 8c,d). The high temperature four-parameter version of the Sharpe-Schoolfield model was applied to development rate and

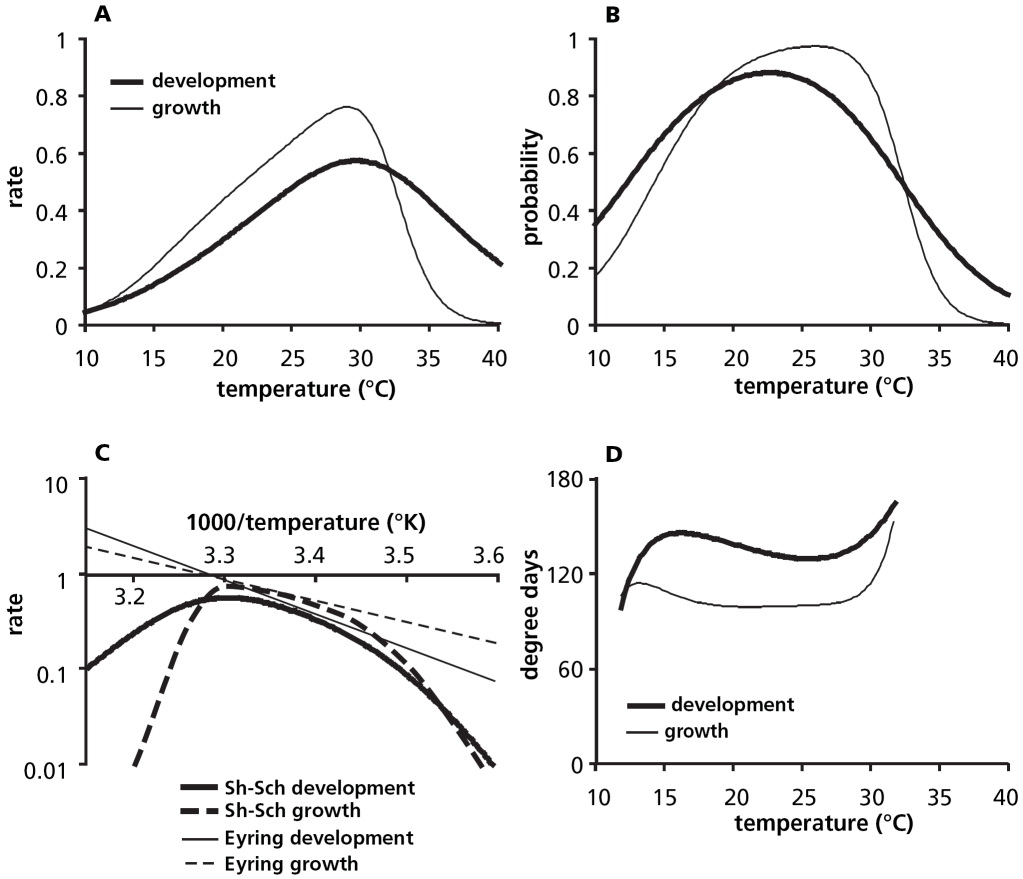

Fig. 7. Rates and probabilities in *Drosophila melanogaster*. Data David & Clavel (1967). Estimates of parameters for development rate are given in Table 2, for growth rate in Table 3. **A**. Development and growth rates and temperature. **B**. Probabilities for developmental and growth enzyme to be active. **C**. Arrhenius plot of ln rate versus 1/T, both according to the Sharpe-Schoolfield equation (Sh-Sch) under estimated parameters, and according to the Eyring equation under estimated parameters. **D**. Degree-day plots for development and growth.

growth rate of four other *Drosophila* species, two tropical species and two temperate (Tables 2 & 3). In all these cases, the experiment itself has not been replicated, and experimental error is therefore unknown. Nevertheless, the order of magnitude of the parameter values is now established for *Drosophila*.

One clear result is that whenever all six parameter values can be estimated, the probability for the rate-controlling enzyme to be active proved never to reach 100%; a maximum enzyme activity might be near to 100% but is not consistently maintained over a range of temperatures (Figs. 8c,d). According to these data there is no temperature at which all enzyme molecules are active; reversible enzyme inactivation is present at all temperatures, and the biological rates are never completely ruled by the Eyring equation and exponentially increasing. Accordingly, development rates seem fairly linear over the middle temperature range in these data, whereas linearity of development rate is impossible with full enzyme activity over a large part of the temperature range: a probability of 100% for the control enzyme to be active results in an exponential increase in development rate. As the development rate approaches linearity over the middle temperature range, the degree-day model is approximately valid. For *D. simulans*, degree-days over 15ºC to 27ºC range from

121-136 (mean 130) in the population from Adana to 132-145 (mean 138) in the population from Houten. For *D. melanogaster*, the degree-days between 15ºC to 27ºC vary from 121-136 (mean 131) in the Asana population to 130-146 (mean 140) in the Houston population. The conclusion is that the degree-day model and the Sharpe-Schoolfield model are compatible over the middle temperature range of the reaction norm for development rate.

Table 2. Parameter estimates of development rate in *Drosophila* species.

Species [1]	Sex, population	ρ. [2]	H_A	T_H	H_H	T_L	H_L
D. melanogaster	David & Clavel	0.567	66,064	32.3	220,632	12.3	- 176,707
D. melanogaster	male, Houten	0.487	73,580	31.6	355,904	11.3	-318,001
D. melanogaster	male, Adana	0.530	86,868	30.3	244,182	13.5	- 1,138,734
D. melanogaster	female, Houten	0.498	74,274	31.9	318,775	11.6	-366,259
D. melanogaster	female, Adana	0.5 10	82,772	30.8	272,655	13.5	- 1,114,203
D. simulans	male, Houten	0.507	52,262	32.4	371,974	13.1	-210,782
D. simulans	male, Adana	0.584	83,441	30.6	271,521	11.0	-365,652
D. simulans	female, Houten	0.500	49,204	32.1	556,844	13.1	-238,509
D. simulans	female, Adana	0.586	82,508	30.8	300,512	11.1	-382,167
D. ananassae	male	0.3378	80,290	31.5	341,733		
D. ananassae	female	0.3400	77,163	31.8	362,784		
D. willistoni	male	0.3258	86,402	28.9	339,119		
D. willistoni	female	0.3440	86,239	28.8	345,762		
D. funebris	male	0.2985	99,242	26.2	260,893		
D. funebris	female	0.2900	96,628	26.7	267,376		
D. subobscura	male	0.2560	69,957				
D. subobscura	female	0.2550	70,244				

[1] First row: *D. melanogaster* as estimated by Van der Have and de Jong (1996) on data from David and Clavel (1967). *D. melanogaster* and *D. simulans* Houten, The Netherlands, and Adana, Turkey, populations data by Jeroen Bohré (unpublished data student graduation project, G. de Jong's lab); estimations based upon 10 vials. *D. ananassae*, *D. willistoni*, *D. funebris* and *D. subobscura* from (Gibert and de Jong 2001); estimation based upon 20 vials. All data apart from David and Clavel's on same fly food in the same lab.

[2] ρ. in 10^{-2} h^{-1}; H_A, H_H, and H_L in J mol^{-1}; T_H and T_L in ºC.

Sharpe-Schoolfield Model and Degree-Days

Development rates might seem fairly linear over the middle temperature range, and the degree-days model is sufficiently experimentally supported, although any increase in development rate might seem linear to a sufficient approximation due lack of statistical power. On the other hand, the Sharpe-Schoolfield model provides a good fit of development rate over the total temperature range. The question arises whether and why empirical parameter values found for the Sharpe-Schoolfield model give rise to approximate linearity over the middle temperature range, and therefore to the degree-day model. One possible approach is to look at the degree-days that would result from the observed parameter estimates in *Drosophila* spp. (Table 2).

Development rates, as in the Houten and Adana populations of *D. melanogaster* and *D. simulans*, were given in Fig. 8a. As the development rate is known for each temperature, the

number of degree-days can be computed. The number of degree-days is relatively constant, at about 90 to 100 dependent upon the *Drosophila* line. Variation in number of degree-days between 15ºC and 27ºC leads to a coefficient of variation of an order of magnitude (4%) that might pass muster in experimental studies as indicating validity of the degree-day model.

Table 3. Parameter estimates of growth rate in *Drosophila* species.

Species [1]	Sex, population	ρ.	H_A	T_H	H_H	T_L	H_L
D. melanogaster	David & Clavel[2]	0.667	39,603	32.4	578,105	14.3	-248,771
D. melanogaster	male, Houten[2]	0.00400	59,446	31.5	448,098	12.4	-351,301
D. melanogaster	male, Adana[2]	0.00400	66,760	31.2	288,458	13.7	-860,398
D. melanogaster	female, Houten[2]	0.00690	71,906	31.4	435,743	12.6	-335,092
D. melanogaster	female, Adana[2]	0.00610	72,459	30.9	471,114	13.7	-719,711
D. simulans	male, Houten[2]	0.00424	73,484	31.3	340,266	10.2	-345,749
D. simulans	male, Adana[2]	0.00430	77,136	30.5	297,395	10.4	-437,998
D. simulans	female, Houten[2]	0.00590	69,099	31.5	726,660	11.3	-290,767
D. simulans	female, Adana[2]	0.00730	99,638	30.5	358,431	11.6	-613,751
D. ananassae	thorax male[3]	3.4930	83,054	30.4	339,826		
D. ananassae	thorax female[3]	3.8483	81,790	30.8	34,652		
D. willistoni	thorax male[3]	2.8473	83,770	28.6	357,922		
D. willistoni	thorax female[3]	3.4401	92,798	27.8	325,489		
D. funebris	thorax male[3]	4.1904	103,583	25.3	285,400		
D. funebris	thorax female[3]	4.6264	101,733	25.4	27,145		
D. subobscura	thorax male[3]	2.7515	66,302				
D. subobscura	thorax female[3]	3.0947	66,563				
D. ananassae	wing male[3]	4.8448	73,526	30.8	343128		
D. ananassae	wing female[3]	5.4173	72,606	30.0	354770		
D. willistoni	wing male[3]	4.4513	75354	28.5	326061		
D. willistoni	wing female[3]	5.1449	76,817	28.3	389725		
D. funebris	wing male[3]	6.5630	95,011	26.5	281633		
D. funebris	wing female[3]	7.1903	94,747	25.5	271604		
D. subobscura	wing male[3]	4.5094	61,830				
D. subobscura	wing female[3]	4.4958	62,267				

[1] First row: *D. melanogaster* as estimated by Van der Have and de Jong (1996) on data from David and Clavel (1967). *D. melanogaster* and *D. simulans* Houten and Adana populations data by Jeroen Bohré (unpublished data student graduation project, G. de Jong's lab); estimations based upon 10 vials. *D. ananassae, D. willistoni, D. funebris* and *D. subobscura* from (Gibert and de Jong 2001); estimation based upon 20 vials. All data apart from David and Clavel's on same fly food in the same lab.

[2] ρ. in mg h^{-1}; H_A, H_H, and H_L in J mol^{-1}; T_H and T_L in ºC.

[3] ρ. in 10^{-3} mm h^{-1}; H_A, H_H, and H_L in J mol^{-1}; T_H and T_L in ºC.

Perhaps the *D. simulans* model-fitting and *D. melanogaster* Adana model-fitting show as good a linear increase in development rate as any set of experimental data. The model for the *D. melanogaster* Houten population shows a curve that looks somewhat more exponential. These curves are estimated from development times and show a middle part as linear as the

Sharpe-Schoolfield model seems capable of. At least, it is difficult to find better linearity by adjusting the model.

Both the Sharpe-Schoolfield model and the degree-day model might be regarded as curve fitting exercises rather than a description of some biological reality. If we take the parameters in the Sharpe-Schoolfield model as representing biological reality, we have to face the question why the actual parameter combinations lead to an increase in development rate with temperature that is near linear. The estimated parameter values for H_H and H_L were relatively low in absolute value, leading to a gradual change in probability that the rate-controlling enzyme would be active. Less enzyme inactivation and a larger range of full enzyme activity is possible with higher absolute values for H_H and H_L. We have to face the conclusion that maximal enzyme activity over a fairly extended temperature range is not present in these data. The prevalence of linearity in the development rate and the applicability of the degree-day model argue against 100% enzyme activity. Maximal enzyme activity over an extended temperature range would lead to an exponential development rate.

If linearity in development rate rather than maximal enzyme activity is selected for, selection might be for constant physiological time. Van Straalen (1983) noted that linearity of development rate with temperature and constant number of degree-days implied a direct transformation from physical time to physiological time. Above, we have argued that a possible biological equivalent of physiological time might be cell number. Functioning of the organism at all temperatures might well involve selection to keep cell number as constant as possible in development. Selection on enzyme inactivation would be a consequence of selection on developmental homeostasis, rather than a cause of differences in development rate.

We make two steps in reasoning here. Both steps start with the observed quasi linearity in development rate. The first step concerns the Sharpe-Schoolfield model. The estimated parameters of the Sharpe-Schoolfield model show that approximate linearity of increase in development rate with temperature corresponds to probabilities of less than one for the control enzyme to be active, throughout. The second step concerns the relation between linearity of development rate and physiological time (van Straalen 1983, BOX 1). If we take the two steps together, physiological time is kept constant if an organism performs at less than 100% enzyme activity at all temperatures, and rates are never constrained by the Eyring equation. If so, organisms adapt to temperature but neutralize its effect by manipulating enzyme inactivation. At 100% enzyme activity, temperature sensitivities of reaction rates rule organismal properties. With enzyme inactivation, the organismal properties overrule environmental temperature influences

Rates and Size: from Sharpe-Schoolfield Model to van der Have Model

The Sharpe-Schoolfield model is usually applied to development rate. In the model description, the temperature dependent rate *r(T)* is specified by the reference rate ρ. The units used in the definition of ρ determine what the rate is about – the units of the rate *r(T)* are those of the reference rate ρ. For development rate, ρ has the unit per time (per hour in Table 2, per day in van der Have and de Jong 1996). The Sharpe-Schoolfield model can be immediately applied to growth in biomass, if ρ is defined as biomass per time, or to growth in length, if ρ is defined as length per time. The temperature sensitivity coefficient H_A,

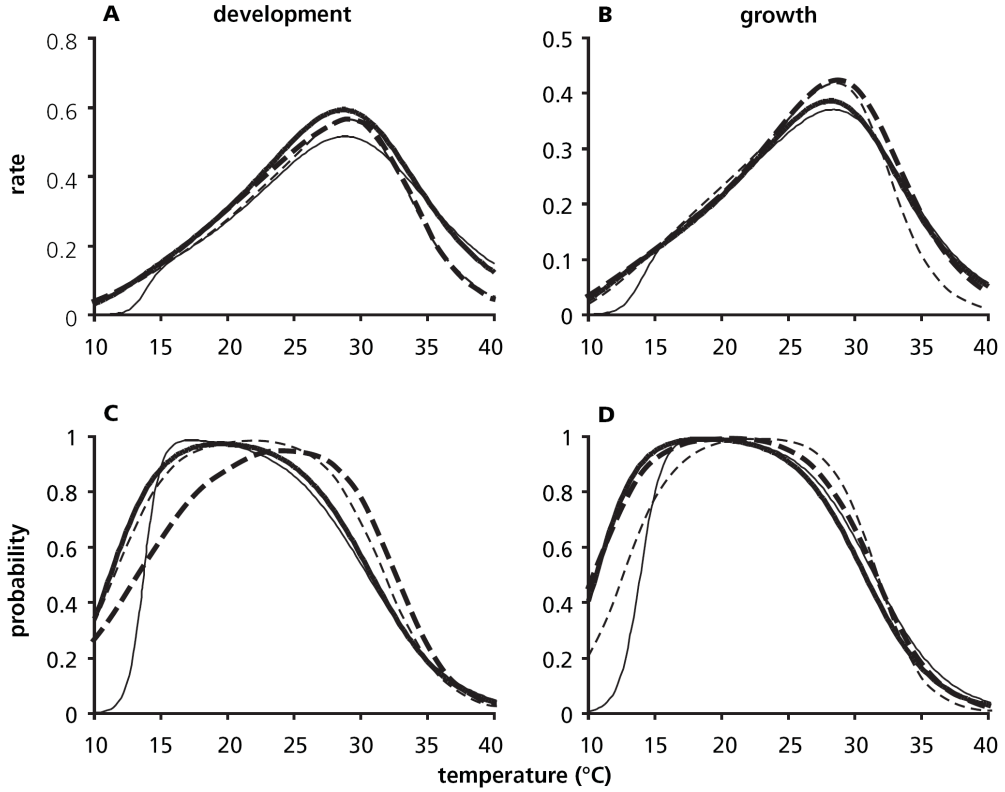

Fig. 8. Rates and probabilities in *Drosophila melanogaster* (thin lines) and *D. simulans* (thick lines), for the Adana (Turkey, continuous lines) and Houten (The Netherlands, broken lines) populations (data by Jeroen Bohré). Estimates of parameters for development rate are given in Table 2, for growth rate in Table 3. **A**. Development rate. **B**. Growth rate. **C**. Probability for the enzyme to be active: development. **D**. Probability for the enzyme to be active: growth.

temperature inactivation coefficients H_H and H_L , and boundary temperatures T_H and T_L are numerically different between growth rate and development rate, but not in units used (Joule per mol, and degree Kelvin). The parameters of growth rate and development rate are indicated by the index G for growth rate and index D for development rate. The parameters for growth rate and the parameters for development rate are assumed to be fully independent. That is, the biological processes for development rate and biomass increase are supposedly different.

The discussion of the properties of the model will take this independence as its starting point, but estimation of parameters introduces inevitably a link. Growth rate can only be estimated from biomass accrued over time, and the time itself is therefore implicated in growth rate.

Van der Have and de Jong (1996) posited that adult size could be found as the ratio of growth rate and development rate. Dividing a temperature dependent growth rate (units mass/time) by a temperature dependent development rate (unit 1/time) yields a temperature dependent adult body size:

$$m(T) = \frac{\rho_G}{\rho_D}\frac{P_G}{P_D}\exp\left[\frac{H_{AG} - H_{AD}}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right] \qquad \text{Equation 6}$$

The temperature dependence of body size depends upon two features: upon the relation between the temperature sensitivity coefficients H_{AG} and H_{AD}, and upon the relation between the probabilities for the enzymes for growth and development to be active. The relative temperature sensitivities of the rates if the control enzymes are active and the probabilities for the control enzymes to be active both influence body size. At high probability of the control enzymes to be active or at equal probability of the control enzymes to be active, body size depends upon the difference in the temperature coefficients H_{AG} and H_{AD}. If P_G and P_D both equal one, the temperature dependence of body size fully depends upon the difference in the temperature sensitivity coefficients H_{AG} and H_{AD}. Lower probabilities for the reaction-limiting enzyme to be active might lead to complications, if these probabilities P_G and P_D differ. If growth rate is more temperature sensitive than development rate, the difference H_{AG} - H_{AD} is positive, and body size increases with temperature. If development rate is more temperature sensitive than growth rate, the difference H_{AG} - H_{AD} is negative, and body size decreases with temperature. Constancy of body size over temperature implies that the temperature sensitivity coefficients H_{AG} and H_{AD} are equal, while the probabilities for the enzyme to be active P_G and P_D are equal too.

Equality of the corresponding parameters in growth rate and development rate might mean one of two things. Development and growth are either biologically identical, or growth is strongly temperature compensated. The first possibility would imply that the increase in biomass and the developmental program of the animal are identical – that development has no features other than biomass increase. This cannot be so, as development entails differentiation next to biomass increase. The second possibility is very interesting. Temperature compensation implies that biomass is selected to remain constant over temperature: the temperature sensitivity of growth rate would be selected to compensate the temperature sensitivity of development rate, leading to a very similar organism at all temperatures. In the data of Gibert and de Jong (2001), the differences between H_{AG} and H_{AD} for thorax size are very small (compare Table 2 with Table 3), and thorax size for the four *Drosophila* species is nearly temperature-compensated.

A decrease of body size with temperature might also result from a decrease in the ratio P_G/P_D (Equation 6). Depending upon the parameter values, this might prevail even if the difference H_{AG} - H_{AD} is positive. In the data of Gibert and de Jong (2001), the difference H_{AG} - H_{AD} is always negative for wing size, and negative for thorax size in male *D. subobscura* and *D. willistoni* (Fig. 9a), but this difference is positive for *D. funebris* (Fig. 9b) and *D. ananassae* (Fig. 9c). The lines marked 'Eyring' in Figs. 9a,b,c indicate how thorax size (or wing size) would change with temperature if the ratio of the probabilities P_G/P_D equaled 1. Actually, all these ratios differ from 1 at higher temperatures (at lower temperature probabilities could not be estimated: this is a 4 parameter version of the model). The decreases in the ratios P_G/P_D are shown in Fig. 9d. The actual thorax size decreases with temperature, but this is completely the consequence of the decrease of the ratios P_G/P_D for these characters and the three species. The lines marked 'Sharpe-Schoolfield' indicate actual thorax size (or wing size), and these lines mirror the probability ratios. In fact, in *D. funebris* (Fig. 9b) and *D. ananassae* (Fig. 9c), the decrease in the ratio overrides the increase in thorax size due to the positive value of H_{AG} - H_{AD}. Maximum thorax size at intermediate temperature might well indicate approximate temperature compensation in H_{AG} - H_{AD}, and a slight difference in the ratio of P_G and P_D towards higher temperature. The decrease of wing size with temperature is a consequence both of negative H_{AG} - H_{AD}, and of a decrease in the ratio P_G/P_D (Fig. 9a,b,c). A decrease in P_G/P_D implies that $P_G < P_D$. This works out to be the

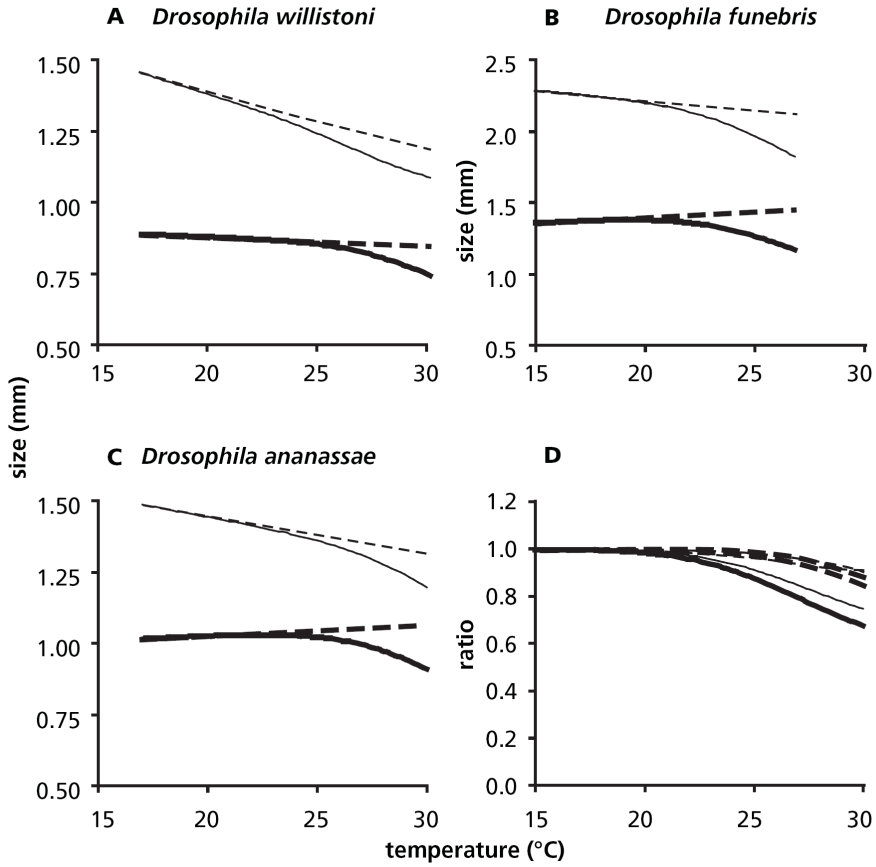

Fig. 9. Body size according to the model of van der Have and de Jong (1996) is given for *D. willistoni*, *D. funebris* and *D. ananassae*. Comparison of the Sharpe-Schoolfield (continuous lines) with the Eyring model (broken lines) shows the influence of the ratio of probabilities, P_G/P_D, on body size, as compared with only the difference H_{AD}-H_{AG} for the Eyring model. The influence of the various parameters differs between species, and between thorax length (thick lines) and wing length (thin lines). All parameter estimates are from Gibert & de Jong (2001), where the observed data for wing length and thorax length are shown. Estimates of parameters for development rate are given in Table 2, for growth rate in Table 3. **A**. *D. willistoni* wing length and thorax length, in mm. **B**. *D. ananassae* wing length and thorax length, in mm. **C**. *D. funebris* wing length and thorax length, in mm. **D**. Ratio P_G/P_D of the probabilities for the rate determining enzymes for growth and development to be active. In *D. funebri*s, the influence of this ratio is high compared with *D. willistoni* and *D. ananassae.*

case in all three species, both for the probabilities for the enzyme to be active estimated for thorax size and for wing the probabilities for the enzyme to be active estimated for wing size.

The actual body size – wing size, thorax size, weight – seems as much or more dependent upon the ratio of the probabilities for the responsible control enzymes to be active than upon the difference between the temperature sensitivity coefficients without enzyme inactivation. Size follows P_G/P_D as much as H_{AG} - H_{AD}, in the few data that are available. In Fig. 10, the body weight is given according to the Sharpe-Schoolfield model including both P_G/P_D and H_{AG} - H_{AD}, and according to the Eyring model just involving H_{AG} - H_{AD}, for the Houten and Adana population of *D. melanogaster* and *D. simulans*. As all reaction norms for development rate and growth rate show quite a linear increase over the middle temperature range, indicating that the probabilities P_G and P_D do not possess a plateau at 100%, this

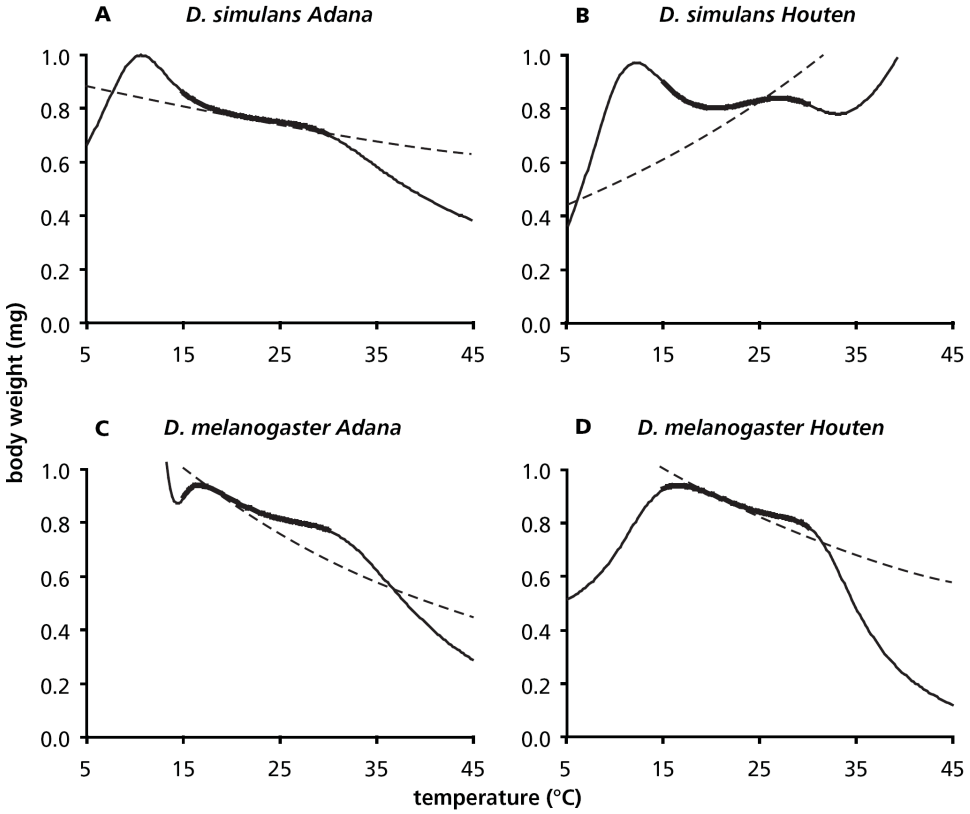

Fig. 10. The model of van der Have-de Jong (1996) is fitted to body size of *Drosophila melanogaster* and *D. simulans*, for the Adana (Turkey) and Houten (The Netherlands) populations. The van der Have-de Jong (1996) model (continuous line) closely follows the data (body weight in mg). Comparison with the Eyring model (broken lines) shows the influence of the ratio of probabilities, P_G/P_D, on body size, as compared with only the difference H_{AD}-H_{AG} for the Eyring model. The influence of the various parameters differs between the species and the populations. Over the viable range 15ºC to 30ºC (thick lines) the influence of the ratio P_G/P_D is less than outside this range. All parameter estimates are from Jeroen Bohré. Estimates of parameters for development rate are given in Table 2, for growth rate in Table 3. Compare Fig. 8 that gives rates and probabilities for the same estimated parameter values for these populations. **A**.*D. simulans* Adana. **B**. *D. simulans* Houten. **C**. *D. melanogaster* Adana. **D**. *D. melanogaster* Houten.

prevalence of P_G/P_D over H_{AG} - H_{AD} is consistent. It is however fairly surprising in its effect. The estimates for the *D. simulans* population form Houten actually indicate a strong conflict between P_G/P_D and H_{AG} - H_{AD} over increase or decrease in size. A decrease in size is not one phenomenon. Rather, the causes of phenotypic plasticity, in terms of the model's parameters, differ with the trait and the temperature range (Angilletta *et al.* 2003). This raises the question as to what actually might be under selection – at least, if we trust the biological reality of the model. If body size itself is under selection, any body size could be reached by any number of parameter combinations. If the parameter values were under selection, body size would follow. This might lead to interesting biological phenomena.

Biological Patterns

We have seen that the Sharpe-Schoolfield model fits data for development rate, and can be used to fit data on development rate and body size. Van der Have and de Jong (1996) and Gibert and de Jong (2001a) estimated parameters, and additional estimates are found in Tables 2 and 3. The model fit works well, but would need additional data, especially data from replicated experiments to determine the experimental error rate.

We will proceed on the assumption that the Sharpe-Schoolfield model for rates and the van der Have-de Jong model for size perform well, in order to examine the possible properties of the model. We know that the upper temperature limit to viability is near the down curve in development rate at high temperature. In the model this down curve is caused by enzyme inactivation. Any potential link between the viability boundaries and the inactivation boundaries needs therefore further examination.

Another point is whether the variation in model parameters can reflect genetic variation in body size within a population. Moreover, we would like to see patterns of body sizes that can conceivably represent a geographic cline in body size within a species, or patterns of body sizes for species with different temperature ranges. In all these cases we only attempt to show the possibilities of the model.

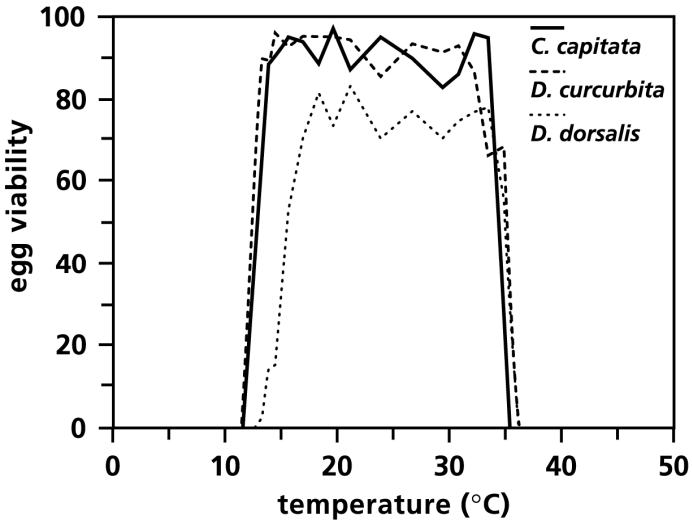

Fig. 11. Temperature viability curves of *Dacus dorsalis, D. cucurbita* and *Ceratitis capitata* illustrate the general pattern of viability boundaries in developing ectotherms. Data are taken from Messenger and Flitters (1958).

Viability boundaries

Temperature-viability reaction norms of ectotherms, including insects, grown at constant temperatures generally have an inverted u-shape (Figs. 3 & 11). The thermal limits of development resemble sharply defined thresholds at high and low temperatures and are symmetrical around the median temperature of viability. The permissive temperature range of embryonic development is usually much narrower compared to the tolerance range of adult physiology like respiration, metabolism in general, or derived performance parameters like running speed or flight speed [fish, Brett (1970); anura, van der Have (2002); *Drosophila*, David *et al.* (1983a)]. It should be noted that the upper thermal limit of development is also much lower than temperatures at which proteins denature irreversibly. To date, few attempts

have been made to explain the threshold character or shape of the viability boundaries and the difference between adult and embryonic performance.

One obvious difference in ectotherms between the adult stage and the embryonic and larval stages in ectotherms is the relative intensity of cell division and differentiation. During development most cells are actively dividing, while in the adult stages cell division occurs mainly in regenerating processes and reproductive tissue not directly linked to performance of the whole organism. This suggests that temperature-induced conformational changes of proteins involved in cell cycle regulation may block cell division and by implication determine the thermal limits of development.

The development of a multicellular organism from zygote to the adult stage proceeds through a series of cell divisions. Cell growth and differentiation are closely co-ordinated with cell division during the larval stage, but are dissociated during embryogenesis. Overall, development can be considered as the interaction between differentiation and growth. Development rate ($time^{-1}$) is assumed to be primarily determined by the cell division rate (van der Have and de Jong 1996).

In this section, a proximate model is presented which shows that temperature inactivation of cell cycle proteins may interact with their regulation and subsequently can predict the temperature tolerance limits of ectothermic development (Van der Have 2002). The analysis suggests that reversible temperature inactivation at high and low temperatures has a symmetrical, inhibiting effect on the balance between synthesis and degradation of cell cycle proteins, resulting in sharp thresholds at the high and low temperatures, above and below which the cell cycle becomes arrested and development blocked. Observed viability boundaries will be compared to thermal limits predicted by the model and derived from differentiation rate - temperature reaction norms in fourteen insect species taken from literature data.

Reversible inactivation of cell cycle proteins. During division, each cell proceeds through a sequence of well-defined stages, together known as the cell cycle. The cell cycle is regulated by the interactions of the subunits Cdc2 and cyclin of the heterodimer MPF (Maturation Promoting Factor) and various cell cycle enzymes (Murray 1992, 1994), Tyson 1991). One of the main goals of this tight regulation is to ensure that the DNA is duplicated exactly once and only once. Several detailed mathematical models of the cell cycle have been developed (Goldbeter 1991, Novak and Tyson 1993a, Novak and Tyson 1993b, Norel and Agur 1991). These quantitative models can quite precisely explain the oscillator phenomena in early embryos and switch mechanisms in growth-controlled cell cycles.

Reversible inactivation of cell cycle enzymes will slow cell division down at low temperatures as well as decrease it at high temperatures. Furthermore, when all enzymes involved will be reversibly inactivated, a gradual response of the whole system can be expected, not the switch-like behaviour of the developmental tolerance limits we are pursuing to explain.

A simple model of derepression as a control mechanism for the cell cycle in eukaryotes was developed by Tyson and Sachsenmaier (1979). They showed how a genetic control system can account for the periodic synthesis of a mitotic activator by sequential dosage changes of an early-replicated repressor and a late-replicated inducer. These dosage changes result in periodic switching of the operon from the derepressed to the repressed state and the activator synthesis respectively off and on at the beginning and end of *S*. The Tyson and Sachsenmaier model is relatively simple and involves both protein-DNA (repressor-operon) and protein-protein (repressor-inducer) binding. It therefore fulfils the above stated prerequisite to serve as a starting point for the analysis of the effects of temperature inactivation on proteins regulating the cell cycle.

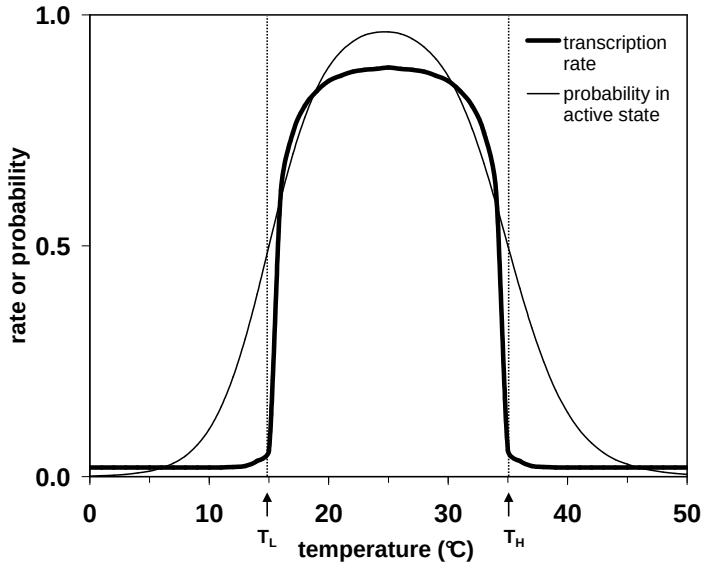

Fig. 12. The probability that the inducer is in active state (P_a) (thick continuous line) and the transcription rate of the genetic operon (thin continuous line) at different temperatures. A steep decrease from near maximum transcription rate to zero transcription rate occurs at temperatures when the inducer (rate-limiting developmental protein) is approximately half active and half inactive (broken line). consequen*tl*y, cell division becomes blocked and development is arrested at the upper (T_H) and lower (T_L) thermal limit.

The following simplifying assumptions have been made for the thermodynamics of the regulation of the cell cycle. (1) The inducer and repressor are proteins, which are assumed to occur in three energy states: active or reversibly inactive at high or low temperature (Sharpe and DeMichele 1977). At high and low temperatures these proteins undergo a conformational transition which renders them inactive with respect to binding properties (Somero 2002). (2) The repressor is more thermolabile than the inducer (Polyak *et al.* 1994), so that the temperature inactivation of the repressor can be ignored over the temperature range at which inactivation of the inducer occurs. (3) The reversible inactivation of the inducer follows Equation 8. (4) The temperature dependencies of all transition rates and equilibrium constants are assumed to be similar, i.e., the rate of inducer degradation is temperature-independent.

Under these assumptions it can be shown (Van der Have 2002) that DNA-replication, and, as a result, cell division will become (reversibly) blocked at the temperature at which the inducer is only half active, while the potential range of biological activity is much wider (Fig. 12). Temperature inactivation of the inducer, therefore, mimics the decrease in inducer concentration resulting from gene dosage changes during the cell cycle. Temperature inactivation of the inducer brings the connection between this model for cell division and the Sharpe-Schoolfield model.

Testing the theory: comparison of predictions with observed tolerance limits. The theory that thermal limits of development are determined by reversible inactivation of cell cycle regulatory proteins can be tested as follows. The temperatures, T_L and T_H, at which 50% of all molecules of a rate-limiting protein or enzyme are reversibly inactivated, can be estimated from temperature-development reaction norms for development rate (see Table 2 for *Drosophila* estimates).

Published datasets of development rates and viability of 14 insect species were used to estimate the thermodynamic parameters from the development rate reaction norm. The estimated values of the 50% inactivation temperatures T_L and T_H from development rate are independent of viability. These temperatures were compared with observed viability curves. An important condition was that the experimental temperatures should cover the full range of viable development (the whole thermal window) for both development rate (embryonic and/or larval) and viability (embryonic or egg-to-adult). The datasets which fulfilled these conditions included eight species of *Drosophila* (Cohet et al 1980; Gibert and de Jong, 2001), three species of *Dacus* fruitflies (Messenger and Flitters 1958), the southern pine beetle *Dendroctonus frontalis* (Wagner *et al.* 1984), and two Homoptera [aphids] *Myzus persicae* and *Lipaphis erysimi* (Liu and Meng 1989).

When the estimated temperatures at which the developmental enzyme has equal probability to be active or inactive at low and high temperatures (T_L and T_H) were compared with the observed thermal limits, 23 out of 25 comparisons (92%) fell closely together (Fig.13). The correspondence at high temperatures is remarkably close in all species. The observed lower tolerance limits (T_L) in *Dacus dorsalis* and *Lipaphis erysimi* do not agree with the observed lower thermal limits, but it should be noted that in these species the estimates for H_A were also outliers. Loss of viability seems therefore the result of too much temperature inactivation of crucial enzymes in cell division – the same enzymes in cell division that are responsible for the temperature dependent development rate.

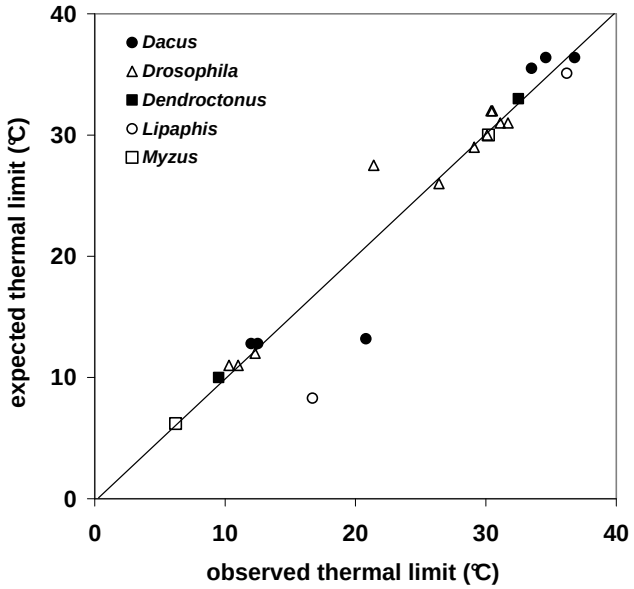

Fig. 13. Observed thermal limits of viability are compared with expected thermal limits of development, T_L and T_H, in fourteen insect species (*Ceratitis*, *Dacus*, *Dendroctonus*, *Drosophila*, *Lipaphis* and *Myzus*) estimated with the Sharpe-Schoolfield model. The equality line ($y=x$) is drawn.

Phenotypic plasticity

The van der Have & de Jong model, ie., Sharpe-Schoolfield model as applied to body size, is eminently suitable to describe phenotypic plasticity of organismal size, if the temperature of larval development determines organismal size. A full model for body size requires estimation

of 12 parameters, 6 for development rate and 6 for growth rate. Development rate and growth rate have a roughly triangular shape unless parameter values are very deviant – unless the probabilities for the control enzyme to be active are very low due to very low absolute values of H_H and H_L. Within the range of parameter values that lead to the classical triangular shape of the reaction norms for growth rate and development rate, many patterns of phenotypic plasticity in body size are possible. The patterns in phenotypic plasticity mostly originate from the ratio of the probabilities for the control enzymes of growth and development to be active; only if this ratio P_G/P_D equals 1 (that includes the case that both probabilities equal 1) does the difference between the temperature sensitivity coefficients of the control enzymes, H_{AG} - H_{AD}, exert a major influence.

In Fig. 14, body sizes are given where the ratio P_G/P_D is changed, by changing either H_{HG} or H_{LG}, or H_{HD} or H_{LD}, while the temperature sensitivity coefficient of growth H_{AG} is kept equal to the temperature sensitivity coefficient of development H_{AD}. This gives a survey of plasticity types for change in one temperature sensitivity parameter superimposed upon a constant body size. The effect of an underlying constant or decreasing body size is given in Fig. 15, where moreover both the high temperature sensitivity parameter and the low temperature sensitivity parameter are changed at the same time.

Body size follows the probability ration P_G/P_D as much as it follows the differences of the temperature sensitivity coefficients H_{AG} - H_{AD}. Any increase or decrease of body size with temperature can have very different combinations of underlying parameters. This means that the possibilities for alternative outcomes in parameter values are very large if body size itself would be under selection. But if the parameters values themselves are under selection – or rather, the biology they might stand for – body size would in many respects be an epiphenomenon to physiological functioning at different temperatures.

Genetic variation

Genetic variation in the parameter values is necessary for selection to have any evolutionary effect. A first indication of the effect of genetic variation in the reference rate ρ, the temperature sensitivity coefficient H_A, and the temperature inactivation coefficients H_H and H_L on (development) rate is given in Fig. 5. Genetic variation in the inactivation temperatures T_H and T_L would lead to a lateral translation of the curves. From genetic variation in parameter values we can infer genetic variation in growth rate, development rate and body size.
A major question is how selection would act. This question has two components. The first question is on what organismal property selection acts: on body size itself, on development rate, on growth rate or on enzymatic properties as temperature sensitivity or inactivation parameters. Often, we cannot ascertain what selection is actually selected on – body size itself, or development rate, or temperature sensitivity of enzymes. This is the question of the nature of the relevant selection pressure. But when thinking in the Sharpe-Schoolfield and Van der Have-de Jong modeling approach, selection on body size translates into selection on parameter values, and selection on parameter values translates into selection on body size. The second question is how selection pressure is translated in a selection response. In general the selection response of any quantitative trait depends upon direct selection mediated by the trait's genetic variance and indirect selection mediated by the genetic covariances with all other traits (BOX 3).

Higher genetic variance or covariance implies faster change in mean phenotypic trait value for identical selection pressures. In the modeling approach, this question translates into the question after the appropriate genetic variances and covariances. What the appropriate

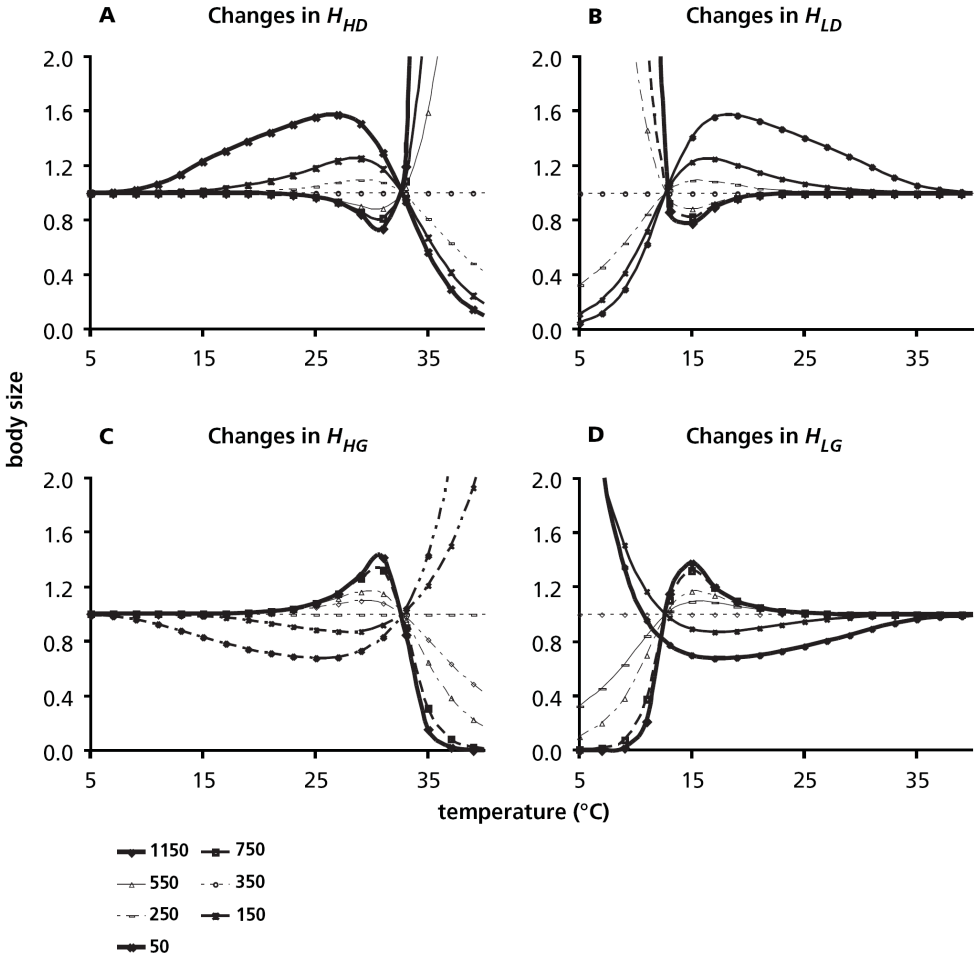

Fig. 14. The influence of the ratio P_G/P_D on body size if either H_{HD}, H_{LD}, H_{HG}, or H_{LG} are changed. In all cases $H_{AD} = H_{AG} = 70$ kJ mol^{-1} and $\rho_D = 0.4$ t^{-1}, $\rho_G = 0.4$ mg t^{-1}: as a consequence, no variation in body size with temperature is expected. The expected body size equals 1 over all temperatures. In all cases T_{ref} = 295.7ºK = 22.5ºC, T_H = 305.7ºK = 32.5ºC, and T_L = 285.7ºK = 12.5ºC. H_{HD}, H_{LD}, H_{HG}, and H_{LG} equal 350 kJ mol^{-1} if H_{HD} or H_{LD} are varied. H_{HD}, H_{LD}, H_{HG}, and H_{LG} equal 250 kJ mol^{-1} if H_{HG} or HL_G are varied between 50 and 1150 kJ mol^{-1}. **A**. Changes in H_{HD}. **B**. Changes in H_{LD}. **C**. Changes in H_{HG} . **D**. Changes in H_{LG}.

genetic variances and covariances are depends partly upon our interest. On the one hand, we might be interested in simultaneous selection at all or a number of temperatures. On the other hand, we might be interested selection at one temperature on body size or some of the model parameters. In all these cases we need to know what the genetic variances and covariances are, specific for each case. In BOX 3, selection is explained, but the selection equations are far more general than the model of body size we are dealing here with and are not necessary for understanding the properties of the biophysical model of insect size.

We will consider how genetic variance in body size is caused by genetic variation in parameter values. Genetic variances in development rate, growth rate and body size will be temperature dependent if genetic variation in the parameters of the Sharpe-Schoolfield model exists in natural populations. Additive genetic variation in parameter values will not only lead to additive genetic variation in development rate, growth rate and body size, but will generate non-additive genetic variation at each temperature too. Both the temperature dependence of

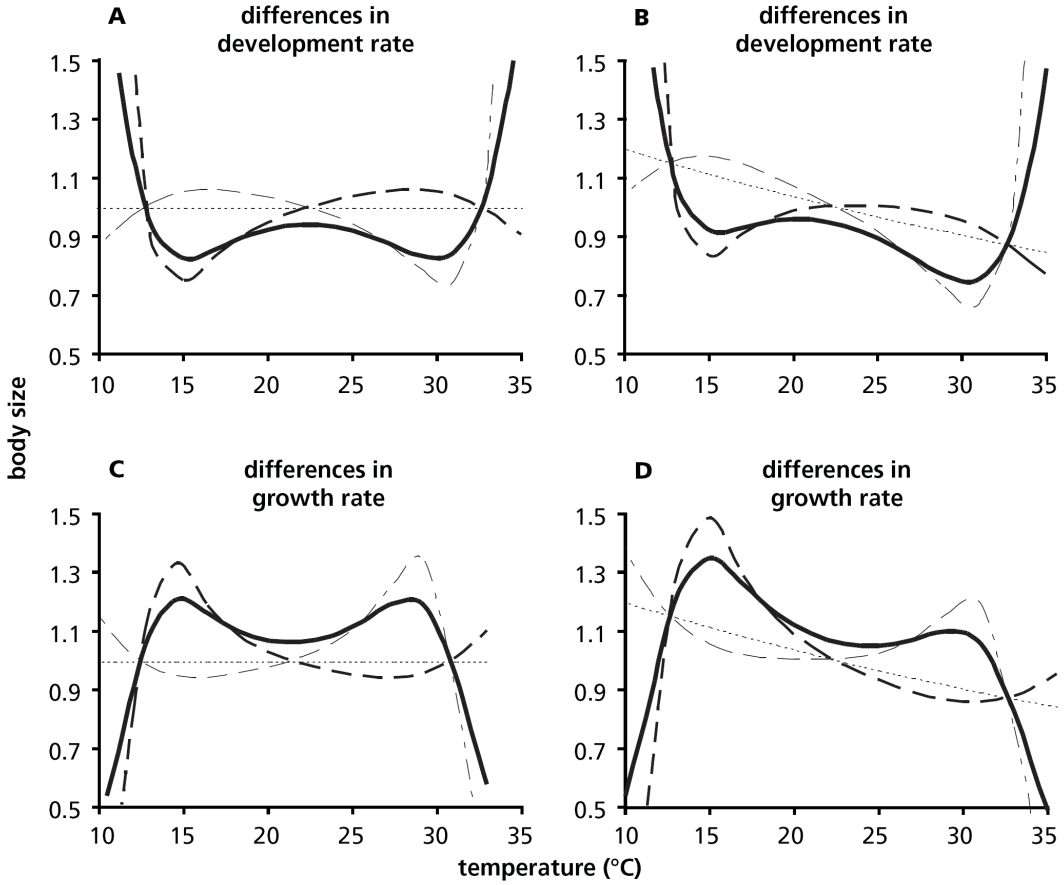

Fig. 15. The influence of the ratio P_G/P_D on body size if H_{HD}, H_{LD}, H_{HG}, or H_{LG} are changed and compared with the Eyring model (broken lines). For basic body weight temperature invariant, $H_{AD} = H_{AG} = 70$ kJ mol^{-1} and $\rho_D = 0.4$ t^{-1}, $\rho_G = 0.4$ mg t^{-1}. If body weight basically decreases with temperature, $H_{AD} = 80$ kJ mol^{-1} and $H_{AG} = 70$ kJ mol^{-1} . In all cases T_{ref} = 295.7ºK = 22.5ºC, T_H = 305.7ºK = 32.5ºC, and T_L = 285.7ºK = 12.5ºC. **A**. $H_{AD} = H_{AG}$, temperature invariant body size, H_{LD} and H_{HD} changed. **B**.$H_{AD} > H_{AG}$, body size decreases with temperature, H_{LD} and H_{HD} changed. **C**. $H_{AD} = H_{AG}$, temperature invariant body size, H_{LG} and H_{HG} changed. **D**. $H_{AD} > H_{AG}$, body size decreases with temperature, H_{LG} and H_{HG} changed. Three combinations are presented: H_{HD} or H_{HG} = 200 and H_{LD} or H_{LG} = 800; H_{HD} or H_{HG} = 500 and H_{LD} or H_{LG} = 500; H_{HD} or H_{HG} = 800 and H_{LD} or H_{LG} = 200.

the genetic variance and the appearance of non-additive genetic variance are a consequence of the non-linear transformations between parameter variation and rate and body size variation.

The temperature dependence of the genetic variance and its subdivision in additive, and non-additive genetic variance components can be studied using a two-locus model. In a two-locus model, additive effects, dominance effects per locus and interaction effects between loci can be estimated if two homozygote lines would be present, and a set crosses involving these lines made. The effects can be estimated from the homozygote line phenotypic values P1 and P2, the mean phenotypic values of the first filial cross F1 and second filial cross F2, and of the backcrosses B1, F1*P1, and B2, F1*P2. The method is involves standard quantitative genetics statistical techniques, and is explained in its application to the Sharpe-Schoolfield model by de Jong and Imasheva (2000).

BOX 3. Selection on body size, selection on parameters

The selection response of a quantitative trait is given by

$$\Delta\bar{z}_i = \frac{1}{\bar{w}}\left(\sigma_i^2\beta_i + \sum_{j\neq i}\sigma_{ij}\beta_j\right) \qquad \text{Equation 7}$$

where $\Delta\bar{z}_i$ stands for the change in mean phenotype of trait *i*, σ_i^2 for the additive genetic variance in trait *i*, σ_{ij} for the additive genetic covariance of traits *i* and *j*, and $\beta_i = \frac{\partial\bar{w}}{\partial\bar{z}_i}$ for the selection gradient of mean fitness $\bar{w}$ towards mean phenotypic value $\bar{z}_i$ of trait *i* (Lynch and Walsh 1997).
Simultaneous selection on *n* quantitative traits can be described by

$$\Delta\bar{\mathbf{z}} = \frac{1}{\bar{w}}\mathbf{G_A}\boldsymbol{\beta} \qquad \text{Equation 8}$$

The column vector $\Delta\bar{\mathbf{z}}$ contains the changes $\Delta\bar{z}_i$ in mean phenotype of the traits, $\mathbf{G_A}$ is the additive genetic variance-covariance matrix with the additive genetic variances σ_i^2 in the traits on the diagonal and the other elements the additive genetic covariances σ_{ij} of traits *i* and *j*, and the column vector $\boldsymbol{\beta}$ has as elements the selection gradients $\frac{\partial\bar{w}}{\partial\bar{z}_i}$ of mean fitness $\bar{w}$ towards mean phenotypic value $\bar{z}_i$ of trait *i* (Lynch and Walsh 1997). Note that the direct selection response in a trait from its additive genetic variance and its direct selection gradient need not have the same sign as the indirect selection response due to the genetic covariances between traits and the selection gradients on all other traits.

Equation 8 is the basic equation. Selection at *n* different temperatures has the same form as selection on *n* different traits; the only complication is that mean fitness over all temperatures is now weighted by how often each temperature occurs. The genetic variance-covariance matrix contains the genetic variance at each temperature, and the genetic covariance between temperatures. Here, we only show how genetic variance in body size depends upon the temperature. In de Jong and Imasheva (2000), a genetic covariance between temperatures is shown too.

The selection response in body size *m* at any constant temperature *T* can be predicted by

$$\Delta\bar{m} = \frac{1}{\bar{w}}\sigma^2\,\beta \qquad \text{Equation 9}$$

Here σ_i^2 is additive genetic variance in body size, and $\beta = \frac{\partial \overline{w}}{\partial \overline{m}}$ is the selection gradient of mean fitness $\overline{w}$ towards mean body size. The selection response in body size can be expressed in the selection responses for the values of all 12 parameters. Call the parameters d_1 to d_{12}. Define three column vectors each with 12 elements. The column vector $\overline{\mathbf{m}}$ has as elements the change in mean body size with mean parameter value, $\frac{\partial \overline{m}}{\partial \overline{d}_i}$; the column vector $\mathbf{\Delta \overline{d}}$ contains the selection responses in mean parameter $\Delta \overline{d}_i$; and the column vector $\boldsymbol{\beta}_{w,d}$ has as elements the selection gradients towards the mean parameter values, $\frac{\partial \overline{w}}{\partial \overline{d}_i}$. The additive genetic variance-covariance matrix $\mathbf{G_A}$ is here the matrix of additive genetic variances and covariances in the parameter values, a 12× 12 matrix.
The predicted selection response in the mean parameter values is

$$\mathbf{\Delta \overline{d}} = \frac{1}{\overline{w}} \mathbf{G_A}\, \boldsymbol{\beta}_{w,d} = \frac{1}{\overline{w}} \mathbf{G_A}\, \overline{\mathbf{m}}\, \beta \qquad \text{Equation 10}$$

with $\beta = \frac{\partial \overline{w}}{\partial \overline{m}}$ as before. This tells us how a selection gradient β on body size is distributed over the various parameters, and how this selection on parameter values themselves leads to a selection response in the parameters. On the other hand, the selection response in body size depends on the selection responses in the parameter values, as

$$\Delta \overline{m} = \overline{\mathbf{m}}^t \ \mathbf{\Delta \overline{d}} \qquad \text{Equation 11}$$

where *t* stands for transpose (i.e. a row vector). Taking Equation 10 and Equation 11 together, we see how change in mean body size derives from selection on the parameter values:

$$\Delta \overline{m} = \overline{\mathbf{m}}^t \ \mathbf{\Delta \overline{d}} = \frac{1}{\overline{w}} \overline{\mathbf{m}}^t\, \mathbf{G_A}\, \boldsymbol{\beta}_{w,d} \qquad \text{Equation 12}$$

In Equation 12, the genetic variance-covariance matrix $\mathbf{G_A}$ between the parameter values does not depend upon temperature, but all other quantities do.

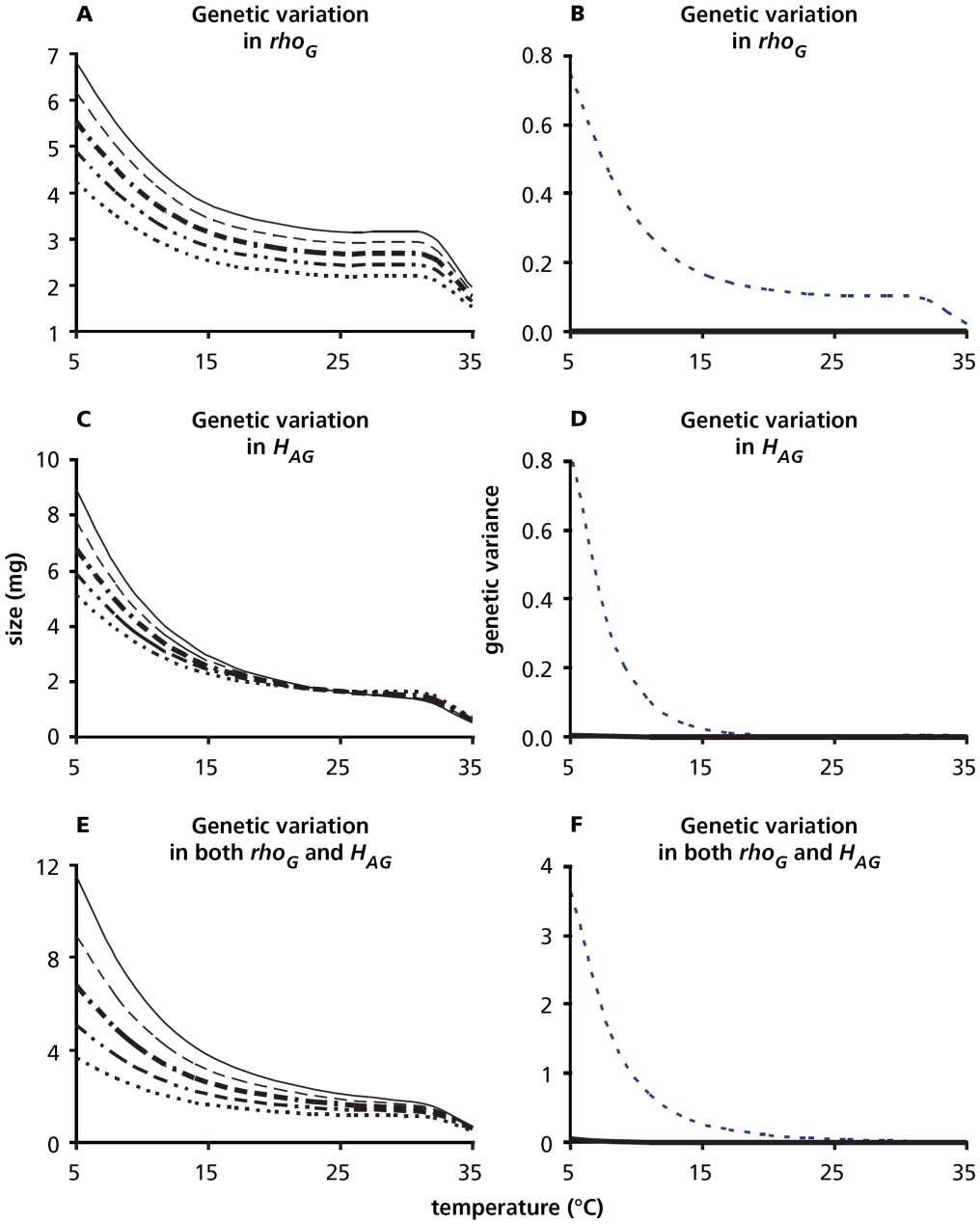

Fig. 16. The effect of genetic variation in reference rate ρ_G and temperature coefficient H_{AG} of growth rate: sizes and genetic variance in body size. Mostly additive genetic variance results from additive genetic variation in the in reference rate and temperature coefficient. The parameter values are based upon the parameter estimates by van der Have and de Jong (1996), based upon *Drosophila melanogaster* data from David & Clavel (1967). See Table 2 for estimates of parameters for development rate and Table 3 for parameter for growth rate. Only mentioned parameter values change, all other parameter values are kept identical. **A**. Nine genotypes at two loci additively differing in ρ_G: ρ_G varies additively from 0. 15 mg t^{-1} to 0.27 mg t^{-1}. **B**. Genetic variance resulting if the allele frequencies at the two loci are p_1=0.6 and p_2=0.3. **C**. Nine genotypes at two loci additively differing in H_{AG}: H_{AG} varies additively from 15 to 35 kJ mol^{-1}. **D**. Genetic variance resulting if the allele frequencies at the two loci are p_1=0.6 and p_2=0.3. **E**. Nine genotypes at two loci additively differing in ρ_G and H_{AG}: ρ_G varies additively from 0. 15 to 0.27, H_{AG} varies from 15 to 35 kJ mol^{-1}. **F**. Genetic variance resulting if the allele frequencies at the two loci are p_1=0.6 and p_2=0.3.

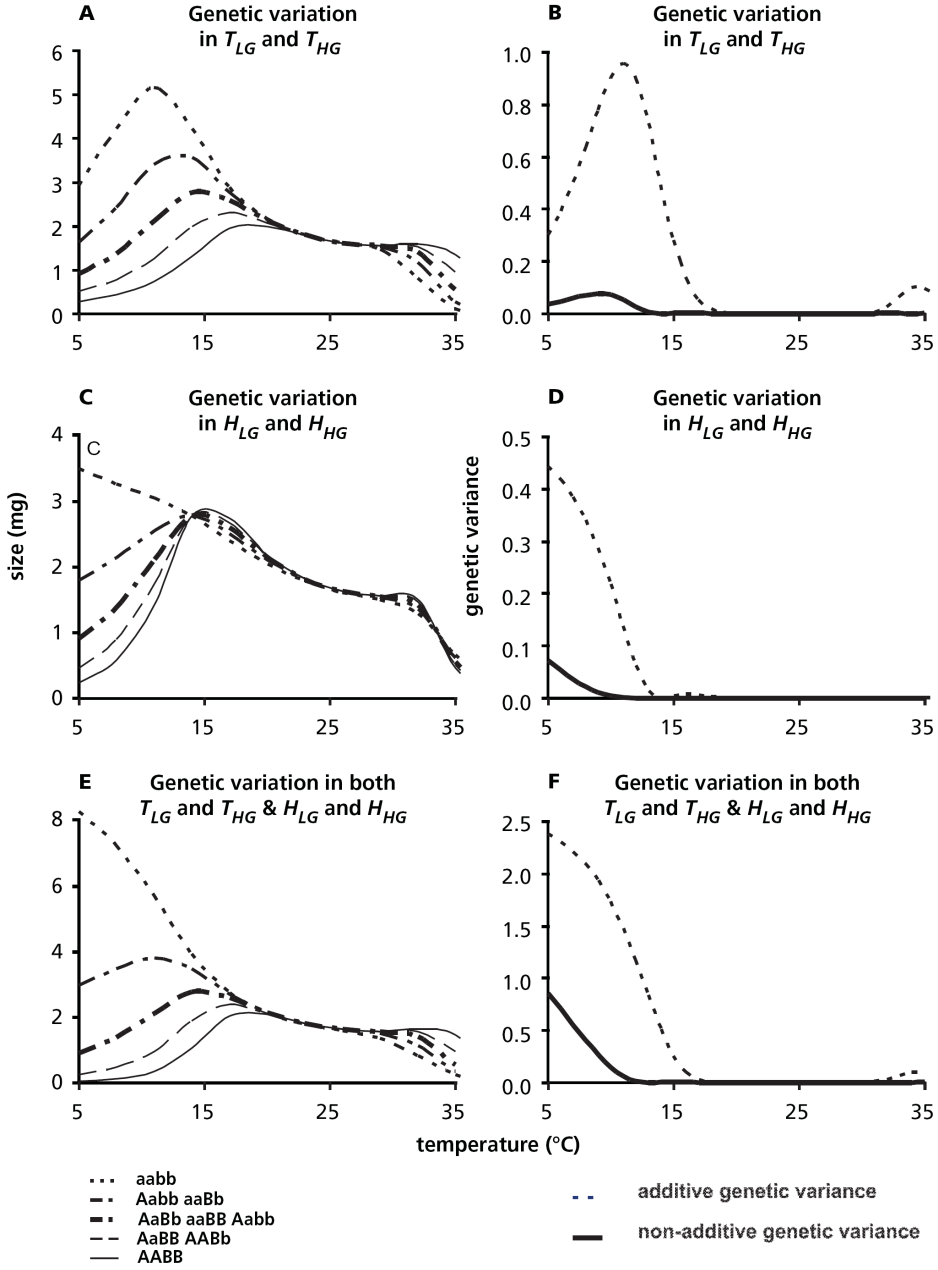

Fig. 17. The effect of genetic variation in temperature boundaries T_{LG} and T_{HG} and temperature sensitivities H_{LG} and H_{HG} of growth rate: sizes and genetic variance in body size. Additive genetic variance results from additive genetic variation in the temperature boundaries and temperature sensitivities, but much non-additive genetic variation is present. The parameter values are based upon the parameter estimates by van der Have and de Jong (1996), based upon *Drosophila melanogaster* data from David & Clavel (1967). See Table 2 for estimates of parameters for development rate and Table 3 for parameter for growth rate. Only mentioned parameter values change, all other parameter values are kept identical. **A**. Nine genotypes at two loci additively differing in T_{LG} and T_{HG}: T_{LG} and T_{HG} vary in concert additively from 285 ºK / 305 ºK (aabb) to 289 ºK / 309 ºK (AABB); T_{ref} does not vary. **B**. Genetic variance resulting if the allele frequencies at the two loci are p_1=0.6 and p_2=0.3. **C**. Nine genotypes at two loci additively differing in H_{LG} and H_{HG}: H_{LG} and H_{HG} vary in concert additively from 900 kJ mol^{-1} / -500 kJ mol^{-1} (AABB) to 500 kJ mol^{-1} / -300 kJ mol^{-1} (aabb). **D**. Genetic variance resulting if the allele frequencies at the two loci are p_1=0.6 and p_2=0.3.

Fig. 17 (continued) **E**. Nine genotypes at two loci additively differing in T_{LG} and T_{HG} and H_{LG} and H_{HG}: T_{LG} and T_{HG} vary in concert additively from 285 ºK / 305 ºK (aabb) to 289 ºK / 309 ºK (AABB), H_{LG} and H_{HG} vary in concert additively from 900 kJ mol^{-1} / -500 kJ mol^{-1} (AABB) to 500 kJ mol^{-1} / -300 kJ mol^{-1} (aabb). **F**. Genetic variance resulting if the allele frequencies at the two loci are p_1=0.6 and p_2=0.3.

De Jong and Imasheva (2000) showed how the genetic variance in development rate and body size over temperature resulted from genetic variation in the inactivation parameters T_L and H_L, and T_H and H_H of development rate. The same method is applied here to make clear possible effects of genetic variation in the parameters of growth rate. In each case, a two-locus model is employed. The parameter values chosen are variations on the values found by van der Have and de Jong (1996) for *Drosophila melanogaster*, but otherwise the values are arbitrarily chosen to demonstrate the ranges of possible behaviors of the model.

In Fig. 16, the effect of additive genetic variation in temperature sensitivity parameters ρ_G and H_{AG} is shown, as well as the effect of genetic variation in both parameters (Figs. 16a, c, and e, respectively). The resulting graphs show that the genotypes differ in size and the additive genetic variance to be temperature dependent (Fig. 16bdf). Non-additive genetic variance is absent if variation is only in the reference rate ρ_G (Fig. 16b) but appears with genetic variation in temperature sensitivity H_{AG} (Figs. 16d,f). Differences in H_{AG} lead to higher genotype by environment interaction than differences in ρ_G: compare Fig. 16c with Fig. 16d. Combination of genetic variation in both parameters leads to a fairly naturally looking set of genotypic values for body size (Fig. 16e), with much higher additive genetic variance than results from variation in just one parameter (Fig. 16f).

Genetic variation in the temperature inactivation parameters T_{LG} and H_{LG}, and T_{HG} and H_{HG} leads to more changes in the shape of the reaction norm of body size, and a more pronounced presence of non-additive genetic variance. Here too natural looking patterns of genetic variation in body size can be modeled. Fig.17a gives the effect of additive genetic variation in the temperature boundaries T_{LG} and T_{HG}, Fig.17b shows the effect of additive genetic variation in the inactivation coefficient H_{LG} and H_{HG} and in Fig.17c variation in all four parameter values is depicted. Of course, genetic variation is mainly apparent at low and high temperatures at the edges of the viable range. The impression would be of increased genetic variation in unfavorable environments (Hoffmann and Merilä1999). Variation in the inactivation parameters leads to more non-additive genetic variance than variation in the sensitivity parameters. This will be the consequence of the position of the inactivation parameters in the denominator of the Sharpe-Schoolfield equation for growth rate. The higher amount of non-additive genetic variance found by de Jong and Imasheva (2000) for genetic variation in the parameters of development rate rather than the parameters of growth rate must be due too to the presence of the development rate in the denominator of body size in the model.

Geographical variation

Geographical variation between populations of the same species implies genetic differences in some of the parameters between the populations. A very interesting source of genetic variation in body size might be genetic variation in the parameters that control the probability for the enzyme to be active, as these parameters are candidates to represent direct adaptation to the environment in enzymatic properties. We might assume that a population that is adapted to a fairly cold environment needs a high probability for the enzyme determining

rates to be active at fairly low temperature, but at the cost of higher enzyme inactivation at high temperature. In such a population, a larger absolute value of H_L would lead to a high probability of the enzyme to be active at low temperature. Relatively low H_H would lead to a certain measure of high temperature inactivation at moderate temperatures. A population adapted to high temperatures might show the opposite pattern in probability for the enzyme to be active – a fairly low absolute value for H_L and a higher H_H. The question is whether such patterns in the parameters are able to mimic actual patterns in body size.

In *Drosophila melanogaster*, body size in temperate populations is larger than in tropical populations (Noach *et al.* 1996, Zwaan *et al.* 2000). The development time shows little or no difference (James *et al.* 1995, James *et al.* 1997) but growth rate differs between temperate and tropical populations (De Moed *et al.* 1998) and increases under cold temperatures in experimental evolution experiments (Robinson and Partridge 2001, Bochdanovits and de Jong 2003).

In keeping with this, we chose an example in which the probabilities for the growth enzyme to be active differ between populations, but everything that has to do with development is the same for the different populations. Five possibilities potentially representing a temperature cline in enzyme parameters within one species are given. The difference between the populations is only in H_{LG} and H_{HG}. Fig. 18 shows the corresponding probabilities for the enzymes to be active (Fig. 18a), growth rates (Fig. 18b), enzyme probability pertaining to development and development rate (Fig. 18c) and body size (Fig. 18d) for the different populations.

The assumed pattern of genetic differences in the temperature inactivation parameters H_{LG} and H_{HG} of growth rate leads to different body sizes in the populations. A higher probability for the enzyme to be active at lower temperature leads to larger body size at lower temperature. The pattern of body size shows a satisfying resemblance to the actual pattern of body sizes laboratory experiments with tropical and temperate populations (for instance, Noach *et al*. 1996).

An important point here is that a parameter representing enzymatic properties is varied, and body size differences follow. This demonstrates that body size itself is not necessarily selected on. The differences in enzymatic properties might be primarily under selection, and body size differences might follow. Alternatively, changes in enzymatic properties might be an efficient way to adapt to the environment if body size itself would be the selected trait.

Of course, clines in body size between tropical and temperature populations might be due to changes in other parameters than the enzyme inactivation coefficients H_{LG} and H_{HG} of growth rate. Genetic variation in the temperature sensitivity coefficient H_{AG} or genetic variation in the reference growth rate ρ_G too might cause a cline. However, changes in H_{LG} and H_{HG} between populations mediate a more intuitively obvious connection to the environment.

Between species variation

Between species differences in body size might result from many different changes in parameter values, but we will concentrate upon one set of parameters that by itself already changes the body sizes – a set of parameters that might not be regarded at first sight as directly influencing body size. In Table 1, the temperature ranges of a number of *Drosophila*

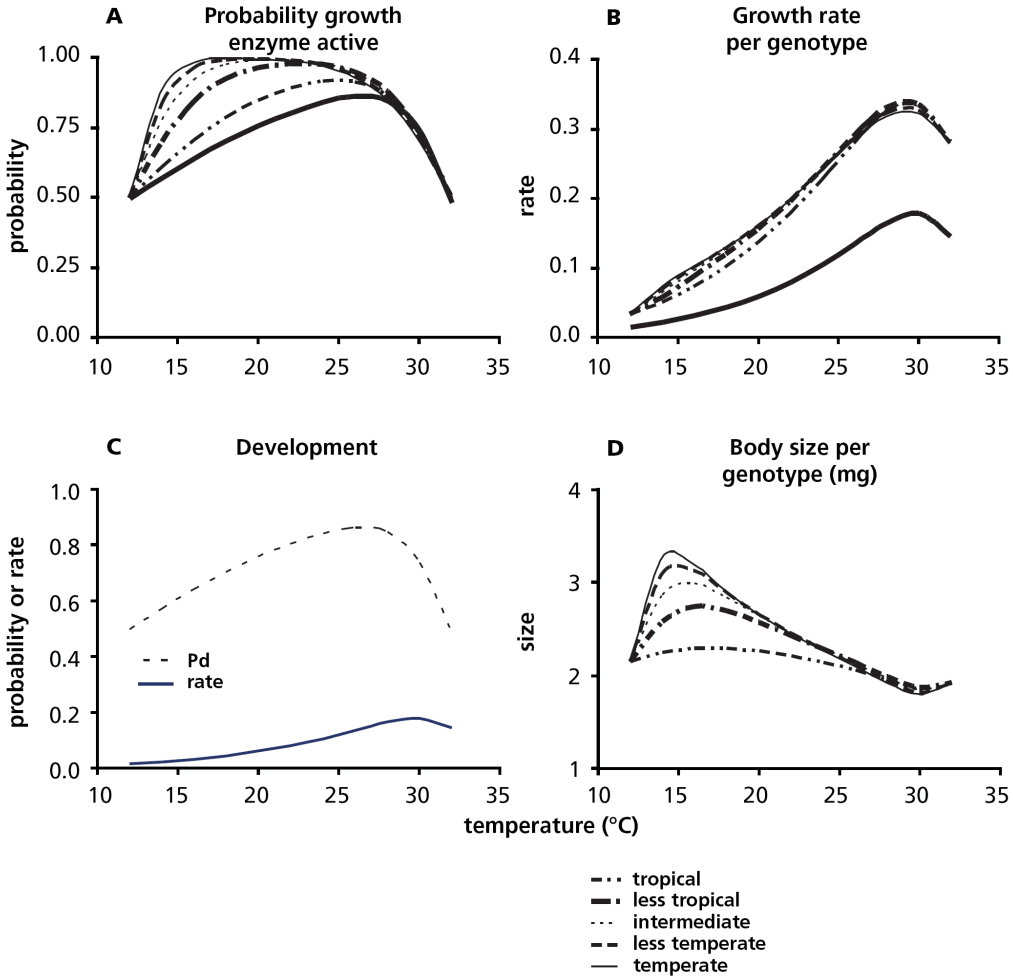

Fig. 18. A cline in body size can be caused by differences in the enzyme inactivation coefficients between populations. Five populations are depicted that differ in the temperature inactivation parameters of growth rate, H_{LG} and H_{HG}. H_{LG} and H_{HG} differ additively and consistently from H_{LG} = -700 kJ mol^{-1} for the temperate population to H_{LG} = - 150 kJ mol^{-1} for the tropical population, and H_{HG} = 325 kJ mol^{-1} for the temperate population to H_{HG} = 400 kJ mol^{-1} for the tropical population. All other parameters are identical between genotypes for the different populations: H_{AD} = 80 kJ mol^{-1}and H_{AG} = 75 kJ mol^{-1}, ρ_D = . 1, ρ_G = 0.2. In all cases T_{ref} = 295ºK = 2 1.8ºC, T_H = 305ºK = 3 1.8ºC, and T_L = 285ºK = 1 1.8ºC. **A**. Probability for the growth limiting enzymes to be active. **B**. Growth rates. **C**. Probability for the development limiting enzymes to be active and development rate. **D**. Body sizes.

species are given. We will concentrate upon the question whether just changing the temperature range but not the sensitivity coefficients or the inactivation coefficients is by itself sufficient to cause a change in body size.

We will model a set of species that are identical in their temperature sensitivity coefficients H_{AG} and H_{AD} for their growth rate and development rate. Moreover, the species are identical in their temperature inactivation coefficients H_{HG}, H_{LG}, and H_{HD}, H_{LD}. The half-way inactivation temperatures T_{HG} and T_{LG}, and T_{HD} and T_{LG}, differ between the species, but are identical for growth and development: $T_{HD} = T_{HG} = T_H$ and $T_{LD} = T_{LG} = T_L$. The reference temperature for each species is chosen exactly at the midpoint between T_L and T_H. The

reference rates ρ_G and ρ_D for each species are found from a reference Eyring equation with given H_{AD} or H_{AG}, and ρ_D =0. 1 and ρ_G=0.2 at *Tref*=295°K. This was done in order to avoid using a reference rate at a reference temperature that is actually outside the enzyme activation range of the species (the equations might not suffer but this way seems more biologically realistic). The growth rates and development rates over the species therefore refer back to one Eyring equation for growth rate and one Eyring equation for development rate. All differences between the species derive from different temperature ranges of enzyme inactivation.

Two different sets of species are compared, both consisting of a graded range from a high temperature adapted species to a low temperature adapted species. In the first comparison, the range T_H-T_L for the enzymes to be active is the same (20ºC) for the five species. In the second comparison, the range T_H-T_L is larger for the cold adapted species than for a species adapted to higher temperature.

Five species differ in temperature boundaries but not in the length of their temperature range. The difference between T_H and T_L equals 20ºC, for both development and growth. The ends of the ranges differ by 1.5ºC between the species. The probabilities for the enzyme to be active are identical, only horizontally shifted by 1.5ºC to different temperatures (Fig. 19a development, Fig. 19b growth). Combined with an identical Eyring equation at different temperature ranges – combining different parameter values for Equation 2 with an identical values in Equation 1 to Equation 3 –, a family of growth rates and a family of development rates originates (Figs. 19c,d). Due to the Eyring equation (Fig. 19e), these rates are not just horizontally translated, but differ in maximum height and slightly in shape. The resulting body sizes are appreciably different, with the species that possesses the lowest temperature range obtaining the highest maximum body size while being smallest at the temperature range the species have in common (Fig. 19f). Maximum body size seems to be related linearly to temperature. The body sizes are translated horizontally by 1.5ºC, and the downward slope over the main temperature range is slightly steeper in the lower temperature species.

A similar but more pronounced pattern is found when the temperature range T_H-T_L is larger for the cold adapted species than for the species adapted to warmer temperatures. This is supposed to be caused by a faster decrease in T_L than in T_H. The probabilities for the enzyme to be active now change in shape, as the width of the temperature range decides whether some sort of plateau in the probability for the enzyme to be active will occur. A low temperature species with a wider temperature range reaches higher enzyme activities (Figs. 20a,b). Development rate and growth rate show more difference between the species, in increase and downturn (Figs. 20c,d). The body sizes differ more than in the case of equal temperature range. Again the species that possesses the range of lowest temperatures obtains the highest maximum body size while being smallest at the temperature range the species have in common (Fig. 20f). The downward slope of body size over the main temperature range is steeper in the lower temperature species. In total, the pattern is similar to but more pronounced than in the case of species with equal width of temperature range.

The importance of this example is as follows: differences in body size are caused by the temperature range, not by any parameter that has to do with growth, development or temperature inactivation. The non-linearity of the Eyring equation implies that no body size based upon a temperature range can be identical to another body size based upon a different temperature range – even if all parameters are other wise equal. One Eyring equation and horizontally translated but identical probabilities for the enzyme to be active do not lead to horizontally translated body sizes of the same shape, but to different reaction norms of body size, of different shapes.

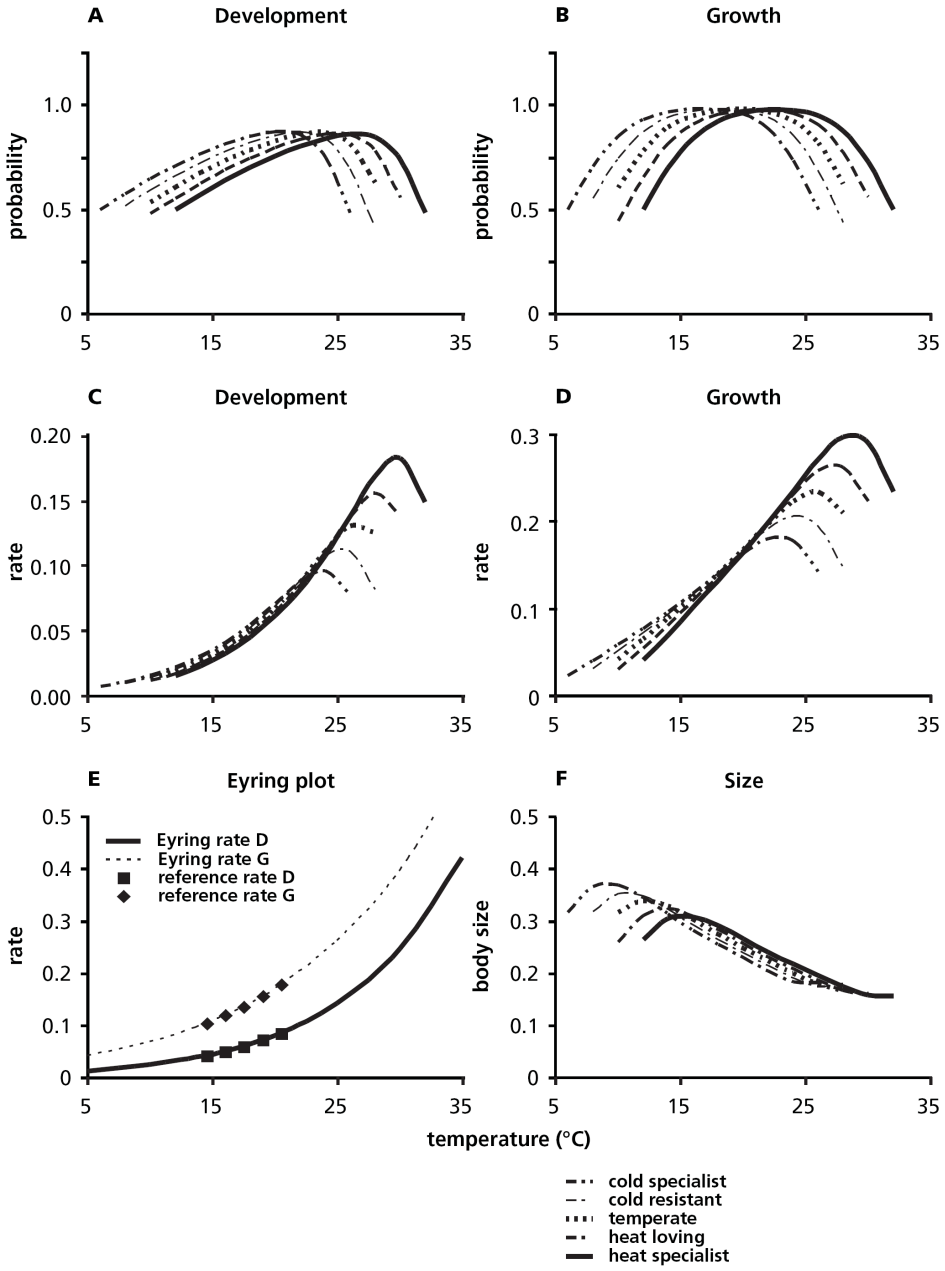

Fig. 19. Between species variation in T_{LD} and T_{HD} , and in T_{LG} and T_{HG} : the range between T_L and T_H equals 20ºC. Five species are depicted ranging from cold specialist to heat specialist. The highest temperature range is 12ºC to 32ºC, the lowest 6ºC to 26ºC. The difference between the species is a 1.5ºC interval. The per species reference temperature is found at the midpoint of the range. The per species reference rate is read from an Eyring equation with ρ_D = 0. 1 t^{-1} and ρ_G = 0.2 mg t^{-1} at T_{ref} = 295ºK. Change of the temperature interval therefore does not imply a change in the Eyring equation in the numerator of the Sharpe-Schoolfield equation. All other parameters are identical between genotypes for the different populations: H_{AD} = 80 kJ mol^{-1} and H_{AG} = 60 kJ mol^{-1}, H_{HD} = 500 kJ mol^{-1} and H_{HG} = 38 1.25 kJ mol^{-1} and H_{AD} = - 100 kJ mol^{-1} and H_{AG} = -287.5 kJ mol^{-1}. **A**. Probability developmental enzyme is active. **B**. Probability growth enzyme is active. **C**. Development rates. **D**. Growth rates. **E**. Eyring plot to find ρ_D and ρ_G. **F**. Body size.

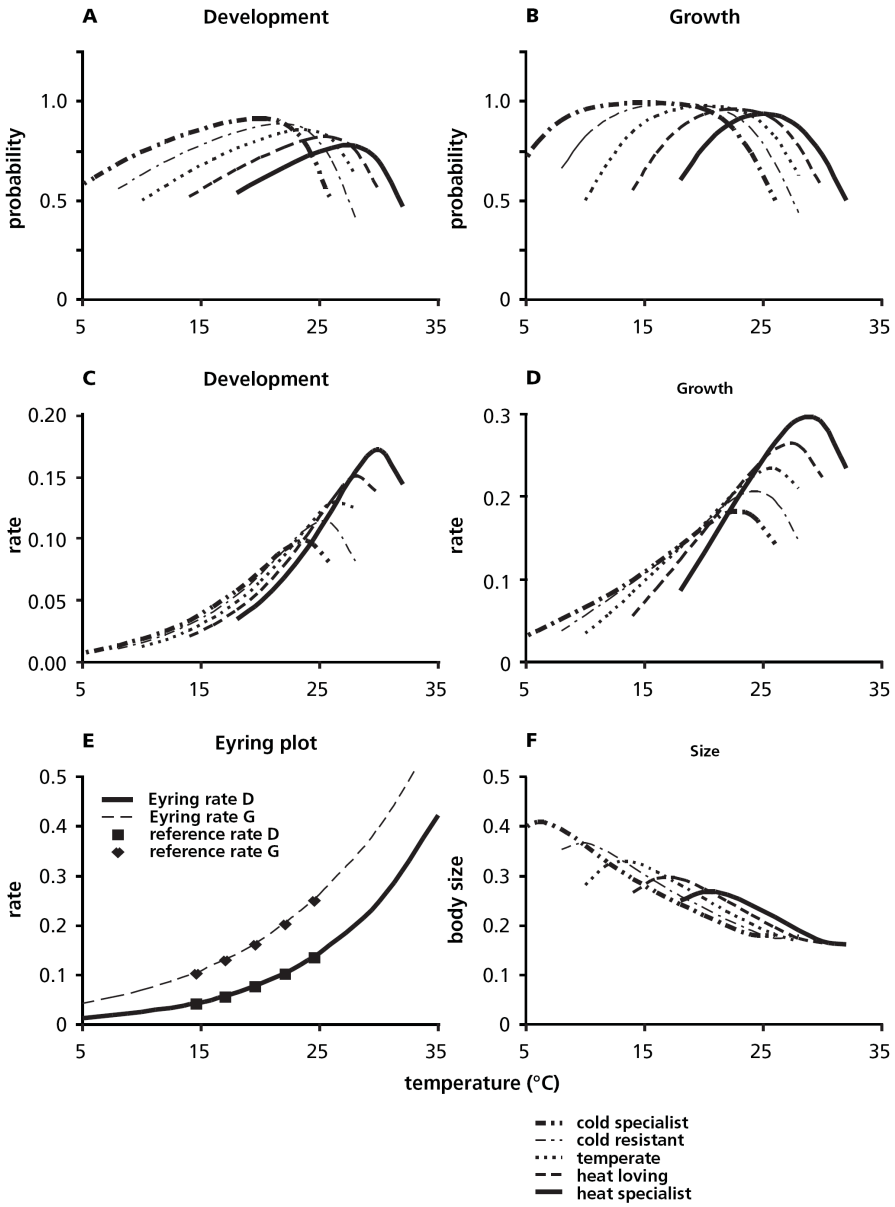

Fig. 20. Between species variation in T_{LD} and T_{HD} , and in T_{LG} and T_{HG} : the range between T_L and T_H increases if the midpoint is at a lower temperature. Five species are depicted. The highest temperature range is 17ºC to 32ºC, the lowest 3ºC to 26ºC. The difference between the species is a 1.5ºC interval for T_H, a 2.5ºC interval for the species specific T_{ref}, and a 3.5ºC interval for T_L. The per species reference temperature is found at the midpoint of the range. The per species reference rate is read from an Eyring equation with ρ_D = 0. 1 t^{-1} and ρ_G = 0.2 mg t^{-1} at T_{ref} = 295ºK. Change of the temperature interval therefore does not imply a change in the Eyring equation in the numerator of the Sharpe-Schoolfield equation. All other parameters are identical between genotypes for the different populations: H_{AD} = 80 kJ mol^{-1} and H_{AG} = 60 kJ mol^{-1}, H_{HD} = 500 kJ mol^{-1} and H_{HG} = 38 1.25 kJ mol^{-1} and H_{AD} = - 100 kJ mol^{-1} and H_{AG} = -287.5 kJ mol^{-1}. **A**. Probability developmental enzyme is active. **B**. Probability growth enzyme is active. **C**. Development rates. **D**. Growth rates. **E**. Eyring plot to find ρ_D and ρ_G. **F**. Body size.

Discussion

How valid or interpretable are biophysical models?

The temperature dependence of biological rates might have been known even before agriculture started, but the earliest cited studies are from 1735 (de Réaumur, as cited by Wang, 1960) and 1855 (de Candolle, as cited by Sharpe and DeMichele, 1977). Both de Réaumur and de Candolle described the degree-day rule. Biophysical descriptions of reaction rates started with the empirical description by Arrhenius. The theoretical derivation by Eyring (1935) and Johnson *et al.* (1974) remains the basis of virtually all further biological descriptions of reaction rates. In Eyring's formulation, any reaction rate scales with temperature T in ºK by a factor $\exp\left[-\frac{H_A}{RT}\right]$, where H_A is the enthalpy of activation and R the gas constant. Eyring's approach must be accepted as standard (Hochachka and Somero, 1984, pg 379-380), and has been indicated as the basis of the temperature dependence of biological rates (Watt 1968, Johnson *et al.* 1974).

Universal Temperature Dependence. Lately, Gillooly and co-authors (Gillooly *et al.* 2001) have developed a theory of the general temperature dependence of biological rates. They aim to document a general exponential relation between rates and temperature within and between species, with one underlying temperature coefficient, in what they call “Universal Temperature Dependence” of biological rates. Gillooly and co-authors write $\exp\left[-\frac{E}{kT}\right]$, with E the Arrhenius activation energy of a biological reaction in eV, and k Bolzmann's constant (Eyring 1935). The Arrhenius activation energy is an approximation of the enthalpy of activation, here called the temperature sensitivity coefficient H_A.

Clarke (Clarke 2004, Clarke and Fraser 2004) criticized an exponential increase of biological rates over species and Universal Temperature Dependence on the basis of the biochemistry of reactions. For one thing, the enthalpy of activation is not the only factor involved in the activation energy, and between species patterns in temperature not only depend upon the enthalpy of activation. For another thing, Gillooly and co-authors' “Universal Temperature Dependence” does not provide any explanation. A number of intertwined patterns were found to be present in the data, but why “Universal Temperature Dependence” should exist is a different question. An explanation in terms of biophysics differs from a statistical description, even if it has the same mathematical form.

We emphasize the importance of parameter variation, and do not subscribe to “Universal Temperature Dependence” as a fundamental constraint. But of more importance here is whether Clarke's criticism of the between species approach of Gillooly and co-authors applies to within species patterns too. The important point is whether biophysical descriptions are compatible with biochemical knowledge of how reactions proceed. Clarke argues that biological reaction rates cannot be exponential with temperature as rates of enzyme catalyzed reactions depend not only on substrate and product but on conformational changes in the enzymatic complex as well. We hope the modification of the Eyring equation to the Sharpe-Schoolfield equation represents the properties of enzymatic complexes adequately for within species purposes. If so, Clarke's criticism is not applicable to the Sharpe-Schoolfield model as used here. The Sharpe-Schoolfield model provides a sufficient statistical description of observed patterns, but we use it mostly as if its parameters provide explanatory variables.

Therefore, we have to be concerned with biochemical adequacy, even if our names for the parameters (temperature sensitivity coefficient, temperature inactivation coefficient) might not be biochemically appropriate.

Linearity of biological rates has been extensively documented (cf Figs. 1-3). Charnov and Gillooly (2003) proposed that this linearity could be regarded as a linear approximation of an exponential increase with temperature. If so, a linear approximation at the reference temperature of the Eyring equation (that is, the numerator of the Sharpe-Schoolfield equation), would have a slope of $\frac{\rho H_A}{RT_{ref}^2}$, in the present notation and in agreement with Charnov & Gillooly (2003). The number of predicted degree-days would be $\frac{RT_{ref}^2}{\rho H_A}$. In the *Drosophila* data, this prediction is nowhere near the actual number of degree-days. In all insect data we have, the linearity of development rate with temperature is clearly not an approximation to an exponential function (Figs. 1-3). The degree-day model depends strictly upon linearity of development rate with temperature. The success of the degree-day model argues against the validity of Charnov & Gillooly (2003)'s approximation: an exponential increase of development rate with temperature gives not even approximately a constant number of degree-days.

The implications of the degree day model. Linearity of development rate requires and implies that in the Sharpe-Schoolfield model the 'probability for the enzyme to be active' never reaches 100%. For direct empirical data, we possess only a limited data set of parameter values. In studies of *Drosophila*, the probability for the enzyme to be active never reaches 100%, and in an Arrhenius plot, a region of strict linearity does not appear. The same observation has been made in other studies using the Sharpe-Schoolfield model, starting with data on plants and insects by Sharpe and DeMichele (1977). Van Straalen (2001) presents data on springtails, and observes the same non-linearity in an Arrhenius plot. The temperature sensitivity coefficient, the enthalpy of activation H_A as used in Sharpe-Schoolfield model, therefore never rules the temperature dependence of biological rates in insects.

Actually, the best evidence for this is the applicability of the degree-day model. The Eyring equation by itself would lead to development rates that are exponential with temperature. Linear development rates imply other processes, and in a model of temperature sensitivity this would require additional parameters. But the most important implication of the linearity of development rate with temperature is the presence of a temperature independent physiological time (van Straalen 1983). As argued above, this physiological time might well be interpreted as constancy of the number of cell divisions. The formal reversible enzyme inactivation in the Sharpe-Schoolfield model would represent the existence of a temperature invariant physiological time, and the regulation of the number of cell divisions. Note though, that the applicability of the biophysical models to temperature sensitivity of biological rates does not depend upon any interpretation of invariant physiological time as a fixed number of cell divisions. Similarly, the van der Have-de Jong model of temperature dependent body size does not depend upon an interpretation of development rate as involving cell number and growth rate as involving cell size.

From biophysical parameters to adaptive patterns in rate and size

Biophysical parameters are not universal constants that rule biology; they are not unavoidable constraints. Temperature dependence abounds, but not equally among species or populations within a species. Yet, given a set of parameter values, temperature dependence of rates is unavoidable. A species' environment will indicate what combination of development rate and growth rate are optimal and in concord with evolved enzymatic parameters. One environment might allow several combinations of rates, sizes and biochemistry to evolve, either as equivalent solutions to the same life history, or as alternative options defining different niches. The temperature dependence of development rate can be used to adjust emergence towards a specific date. Consider for instance a univoltine species with a short mating season in early summer. Eggs might have hatched at different times in spring. The temperature dependent development rate should be selected to synchronize adult emergence and thereby enhance efficient reproduction (Gilbert and Raworth 1996). To function in this way, development rate needs to be temperature dependent, within any specific latitude and between latitudes, but the temperature dependence must be under genetic and evolutionary control.

Generation time relative to season length will decide whether a latitudinal cline in body size will show larger body at higher latitudes or lower body size at higher latitudes (Chown and Gaston 1999). A strictly univoltine species might well show smaller body size at higher latitude as a consequence of shorter season length. Small multivoltine insect species with relatively fast development show larger body size at higher latitudes (Blanckenhorm and Demont 2004). Over species, growth rate and development rate have to respond independently to account for this diversity of observed patterns in insect latitudinal clines.

Adult size is widely regarded as the ratio of growth rate to development rate (Gilbert and Raworth 1996). Temperature compensation in adult size occurs when the effect of temperature dependence disappears due to the division – that is, when the temperature sensitivity coefficients of development and growth H_{AD} and H_{AG} are equal and when the probabilities for the enzyme to be active are equal. In the *Drosophila* data, H_{AD} and H_{AG} differ significantly between species but for thorax, H_{AD} and H_{AG} do not differ significantly. Thorax length might be the most obvious indicator for adult size, and the evidence seems to be that *Drosophila* species almost compensate for temperature dependence in body size. The generally observed decrease in adult size with temperature (Atkinson 1994, 1996) might be restricted to the region of high temperature enzyme inactivation – or be a consequence of experimenter choice of character to measure. Wing size, in contrast to thorax size, decreases steadily with temperature, but this might be as much an adaptation to flight at different temperatures as indicative of a general decrease in body size with temperature (Petavy *et al.* 1997), though the actual decrease in wing size depends upon the population (Noach *et al.* 1996).

In *Drosophila*, the differences in the temperature sensitivity coefficient for development H_{AD} are almost significant (data in Table 2, P=0.087 over 6 species) but the differences in the temperature sensitivity coefficients for growth H_{AG} are significant (data in Table 3, P=0.013 over 6 species for thorax and P<0.001 over 4 species for wing). Larger H_A occurs in the species *D. willistoni* and *D. funebris*. No obvious ecological correlate is evident.

Van Straalen (2001) applied the Sharpe-Schoolfield model to temperature dependent development time in springtails. The model described the data very well. Over 38 species, parameter values for development rate at 15ºC and for temperature sensitivity coefficient H_A were compiled. Springtails can be grouped in ecological classes depending on their place in the leaf litter and soil surface. The epigeon, the group of species that lives on the soil surface, has highest H_A values, significantly different from the values in the euedaphon, the group of species that lives in the soil. Hemiedaphon species that live in the litter layer have

intermediate values for H_A. In van Straalen's study temperature sensitivity of development and ecological niche are clearly related.

From biophysical parameters to adaptive phenotypic plasticity

Different theoretical possibilities for phenotypic plasticity emerge just by changing a few of all the parameters in the model. The most obvious parameters to change to obtain a change in phenotypic plasticity of adult body size are the temperature sensitivity coefficients H_{AD} and H_{AG} (the enthalpy of activation of enzymatic reactions for development and growth). The difference between H_{AD} and H_{AG} is a main factor in deciding plasticity of body size towards temperature, as shown in Fig. 15. But, as is clear too in Fig. 15, it is quite possible to observe phenotypic plasticity in body size of opposite sign to that indicated by the difference between H_{AD} and H_{AG}. The temperature inactivation coefficients (H_{HD} and H_{HG}, H_{LD} and H_{LG}) decide as much about phenotypic plasticity as the temperature sensitivity coefficients.

The patterns of phenotypic plasticity we demonstrate are therefore not derived from difference in the temperature sensitivity coefficients H_{AD} and H_{AG}. Without changing the temperature sensitivity coefficients we simulated temperature dependence of body size. The parameter values we changed have been chosen to indicate how actual patterns of phenotypic plasticity might be formally caused, and we took our cue from observations in *Drosophila*. We concentrated on patterns of differences in phenotypic plasticity that were actually observed, and tried to find biologically plausible but simple ways to generate such patterns. In *D. melanogaster*, clines in allozyme frequency have repeatedly been described (Eanes 1999). In such clines, allozymes with higher enzyme activity might predominate in more temperate populations. For some enzymes, an allozyme with high enzyme activity at crucial points in the metabolic network has relatively higher activity at low temperature and reaches high frequency in temperate populations (Verrelli and Eanes 2001). Relatively high enzyme activity at lower temperature might lead to larger adult flies (Bijlsma-Meeles and Bijlsma 1988). The observation is for the enzyme alcohol dehydrogenase; its generality is unknown.

In Fig. 18, a cline in enzyme activity was simulated, taking our clue from the enzyme clines in *Drosophila melanogaster*. In Fig. 18, a situation is modeled where enzyme activity changes from a maximum at low temperature to a maximum at high temperature. Body size changes with enzyme activity, leading to a larger body size at low temperature for the parameter set with the maximum enzyme activity for growth at low temperature. Of course, many other parameter values can be chosen to represent differences in enzyme activity. Changing from enzyme activity for growth with a low maximum at low temperature to high enzyme activity for growth over a much wider range, for instance results in almost parallel reaction norms for body size over the *D. melanogaster* viable range. We have been able to reproduce the observed patterns of reaction norm comparisons in natural populations by changing the probability of the enzyme for growth to be active.

Larger size at lower temperature resulted from the model equivalent of a higher enzyme activity at lower temperature (Fig. 18). This leaves open what selection pressures would operate in a natural population. Selection could be for larger adult size at lower temperatures, without any selection pressure for populations to differ at higher temperatures. The selection pressure on body size itself could be translated into a selection pressure on enzyme activity at low temperature (see Box 3 how such translation works). Or, selection could directly be on enzymatic functioning, on enzymes that perform better at lower temperatures, and any change in body size itself could be a correlated response. The formal description in terms of the biophysical model would be the same.

Other changes in body size and of phenotypic plasticity in body size can be derived from changing the temperature range of enzyme activity without changing the temperature

sensitivity coefficients (H_{AD} and H_{AG}) or the temperature inactivation coefficients (H_{HD} and H_{HG}, H_{LD} and H_{LG}). This is a particularly interesting observation, as it indicates that many patterns in temperature dependence of adult body size in insects might derive from the temperature boundaries rather than from temperature sensitivity over the viable range itself.

Biophysical models like the Sharpe-Schoolfield model are a fertile field for biological explanation of temperature related plasticity in development rate and body size in insects. At the moment, the number of available parameter estimates is very low, and this prevents us from having insight into the ecological and evolutionary patterns that might be associated with biophysical parameters. We hope it will be clear that the models are applicable and can be used to categorize temperature related ecological differences, as exemplified in the observations of van Straalen (2001) on springtails.

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CHAPTER 5

THE EYRING EQUATION AND THERMAL ADAPTATION IN DEVELOPING ECTOTHERMS

T.M. van der Have and G. de Jong

Abstract

This paper investigates how patterns in thermal adaptation within and among species can be modelled and predicted from the Eyring equation and Sharpe – Schoolfield model which is based on the kinetics of reaction rates and enzyme properties. The slope of within-species linear thermal reaction norms can be approximated by the tangent to the general temperature dependence among species predicted by the Eyring Equation. The linearity itself is caused by reversible temperature inactivation. The thermal time model complies with the linear part of the developmental rate – temperature reaction norm describes by the Sharpe - Schoolfield model.

It is shown that small changes in the parameter variation in the biophysical Sharpe - Schoolfield model can generate all three major patterns of thermal adaptation and in addition a fourth pattern, namely, a sensitivity shift or variation in the slope of the thermal reaction norm. A shift in sensitivity can result directly from variation in reference rate or activation energy, and indirectly from a shift in thermal range or optimal temperature. Therefore, a phenotypic shift in sensitivity is not informative about the underlying change in thermal parameters included in the Sharpe – Schoolfield model.

If only the development rate at the reference temperature is varied, and all thermal parameters are kept constant, the slope of the linear part will vary with the reference rate but the threshold temperature will remain constant. This situation applies to developmental rate isomorphy, the observation that in many insect species the temperature sensitivity varies with developmental stages but the threshold temperature h remains constant. It is proposed that the Sharpe – Schoolfield model provides a mechanistic explanation for developmental rate isomorphy.

Finally, the variation in thermal reaction norms in a large number of anurans based on published datasets is analysed. In about 50 species most thermal parameters, such as reference rate, optimal temperature, thermal range and thermal tolerance limits have been experimentally measured. All four patterns of thermal adaptation occur in this group of related anurans, but that most variation in ρ and slope can be explained by the combination of a horizontal shift (hotter – colder) and a response following the Eyring equation. The implications of the results are discussed in relation to the assumptions of the proposed Universal Temperature Dependence, the correlation between egg size and development rate and the evolutionary optimisation of egg size in relation to thermal environment.

Introduction

Eyring (1935) developed a theoretical foundation in absolute reaction-rate theory for the empirical Arrhenius equation, which describes the exponential temperature dependence of chemical reactions. Sharpe & DeMichele (1979) applied Eyring's theory to a unified rate model that describes the rate of biological processes for all temperatures that support life. Most biological rates do not increase exponentially with temperatures as do chemical reactions, but increase quasi-linearly above a certain threshold up to a maximum rate (Figure 1A). Sharpe & DeMichele (1979) proposed that reversible inactivation at high and low temperatures linearizes the exponential Eyring equation over much of the thermal range and, therefore, provides a mechanistic model for biological rates within species over the total biological range. Schoolfield *et al.* (1981) modified the model of Sharpe & DeMichele (1979) giving the model parameters that have a direct biological interpretation, called the Sharpe – Schoolfield model.

The empirical Arrhenius equation is basically similar to the theoretical Eyring equation. An approximation to the Arrhenius equation was recently used to describe the so-called universal temperature dependence (UTD) of metabolic rate (Gillooly *et al.* 2001) and development time (Gillooly *et al.* 2002). This use of the empirical Arrhenius equation to predict patterns in temperature dependence of metabolic rate among a wide range of species (Gillooly *et al.* 2001) has received considerable criticism (Clarke & Fraser 2004, Clarke 2004, 2006, O'Connor 2007). These critiques have in common that the relationship between temperature and metabolism is considered to be complex and, therefore, any overall description of this relationship should be considered statistical rather than mechanistic.

Charnov & Gillooly (2003) extended the UTD model to the thermal time approach. Thermal time refers to the observation that in many, if not most, ectotherms development rate (1/time) is linearly related to temperature (°C) over much of the thermal range. Charnov & Gillooly (2003) suggested that these linear developmental rate relationships can be considered as linear approximations to the exponential function described by UTD, totally ignoring all entomological literature documenting strict linearity over quite a large temperature range, In fact, Charnov & Gillooly (2003) suggest that the temperature dependence of development rate is essentially the same within and among species In fact, the UTD approach tends to regard the exponential temperature dependence of biological rates as a fundamental fact of nature; a biological explanation of biological rates is secondary to physical constraints. Although Gillooly *et al.* (2001, 2002) suggest that enough species-specific variation exists around the cross-species allometric relationships to be explained in biological terms, the UTD approach clearly accepts a non-biological main effect on biological rates. The UTD lacks a clear link with genetics to explore the evolution of thermal adaptation within and among species. The Sharpe - Schoolfield model is also based on stoichiometric principles and general temperature dependence defined by the Eyring equation, but has a considerable advantage that heritable variation in biochemical properties of enzymes can be included in all of the thermal parameters (De Jong & van der Have 2007, Chapter 4)).

Studies of thermal adaptation usually focus on a relatively small part of the thermal range, such as studies of cold adaptation (Clarke 1991) or heat shock proteins near the upper thermal limit (Feder & Hoffmann 1999). Fewer studies address mechanisms operating at both low and high temperature (Hoffmann *et al.* 2003, Pörtner & Knust 2007, and Van der Have 2001, Chapter 3). On the other hand, models of thermal performance (Huey & Kingsolver 1989, Kingsolver & Gomulkiewicz 2003) seem too general to understand the proximate mechanisms involved in thermal adaptation, although they are suitable for describing the evolution of thermal adaptation (Gilchrist 1995, 1996). Three main patterns of thermal

adaptation operating within species and among populations have been proposed and observed: (1) vertical shift (faster-slower), (2) horizontal shift (hotter – colder) (3) generalist – specialist or change in tolerance range (Huey & Kingsolver 1989, Izem & Kingsolver 2005).

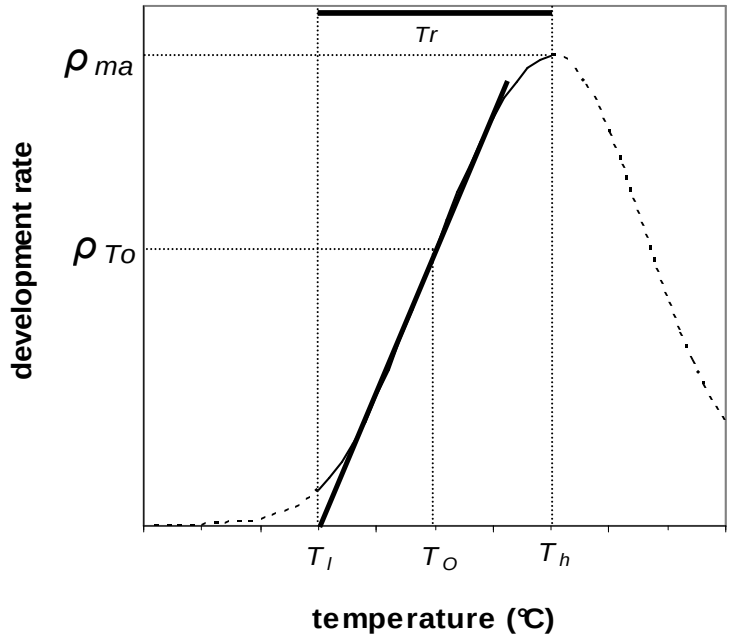

Figure 1. Temperature dependence of development rate is non-linear (dotted line) but well represented by a straight line (continuous line) within the upper and lower limits of development (T_l and T_h respectively). The tolerance range of development (T_r) is defined as the difference between T_l and T_h. The optimal temperature of development (T_o) is defined as the mean of the upper and lower tolerance limits. ρ_{To} is the development rate at T_o and $\rho_{\max}$ is the maximal development rate observed.

A major challenge is to find explanatory models which can be extended from within species- to among-species variation without loss of biological realism. Such a model should preferably based on thermodynamic properties of biochemical reactions and include parameters which can be linked to heritable variation. The Sharpe - Schoolfield model originally proposed by Sharpe & DeMichele (1977), and adapted by Schoolfield *et al.* (1981) to make it more suitable for non-linear regression, fits well to these requirements. Their method has been widely applied in entomology as a non-linear regression method relevant to within species temperature dependence (*e.g.*, Kontomidas 2004) but is less well known outside this field. Recently, the model was extended conceptually to predict the temperature-dependence of body size (also known as the temperature – size rule, van der Have & de Jong 1996 [Chapter 2], de Jong & Gibert 2000, see also Walters & Hassall 2006), thermal tolerance limits (van der Have 2002), genetic variance in adult size (de Jong & Imasheva 2001), and clines in body size (De Jong & van der Have 2007, Chapter 4).

This paper focuses on two questions: (1) how can patterns in thermal adaptation within and among species be modelled en predicted from the Eyring equation and Sharpe - Schoolfield model based on the kinetics of reaction rates and enzyme properties? (2) Are within-species linear temperature-development rate reaction norms simply approximations of a general temperature dependence among species predicted by the Eyring equation?

To answer these questions we will first compare the thermal time model, the Sharpe - Schoolfield model and the Charnov & Gillooly (2003) approach, especially over the linear part of the developmental rate – temperature reaction norm. Secondly, we will show that parameter variation in the biophysical Sharpe - Schoolfield model can generate all three major patterns of thermal adaptation and in addition a fourth pattern, namely, a sensitivity shift or variation in the slope of the thermal reaction norm. Finally, we will analyse the variation in thermal reaction norms in a large number of anurans based on published datasets. In about 50 species most thermal parameters (ρ, T_o, T_r, T_l, T_h, slope, Figure 1) have been experimentally measured. We will show that in anurans all four patterns of thermal adaptation occur, but that most variation in ρ and slope can be explained by the combination of a horizontal shift (hotter – colder) and a response following the Eyring equation.

THE MODEL

In many ectotherms development rate *r(T)* varies non-linearly with temperature (in °C) near the lower and upper thermal limits (T_l and T_h), and linearly over much of the thermal range T_r, of viable development (Figure 1A). We define ρ_{To} as the development rate at the reference temperature T_o, the median between T_l and T_h, and ρ_{max} as the maximal development rate, at T_{max}. If the linear part can be represented by $r(T) = b(T-h)$, where b is the slope of development rate with temperature T (in °C) above a certain threshold temperature h (in °C), then the temperature sum S for development (in degree-days, also known as thermal time) is constant and equals $1/b$ (*e.g.*, Trudgill *et al.* (2005). In the notation of Schoolfield *et al*. (1981) the Eyring equation reads:

$$r(T) = \frac{\rho_{T_o} T}{T_o} \exp\left(\frac{H_A}{R} \left(\frac{1}{T_o} - \frac{1}{T} \right) \right) \qquad (1)$$

Where *r(T)* is the biological rate at temperature T (in °K), ρ_{To} the rate at the reference temperature T_o, H_A the enthalpy of activation (J/mol), and R the universal gas constant (8.314 J K^{-1} mol^{-1}),

Gillooly *et al.* (2001, 2002) start out with a similar equation, but use average energy per reaction $\overline{E}$ and the Boltzmann constant k rather than enthalpy H_A and the gas constant R. The units used change from J/mol to electronvolt eV. Gillooly *et al*'s (2002) starting equation reads

$$r(T) = \frac{\rho_{T_o} T}{T_o} \exp\left(\frac{\overline{E}}{k} \left(\frac{1}{T_o} - \frac{1}{T} \right) \right) \qquad (2)$$

and as both the actual temperature T and the reference temperature T_o are in the range of 280-310 °K, approximations give that

$$r(T) \propto \exp\left(\frac{\overline{E}}{k T_o^2} T \right), \qquad (3)$$

their version of the biological reaction rate. More on biological reaction rates can be found in Hochachka and Somero (1984).

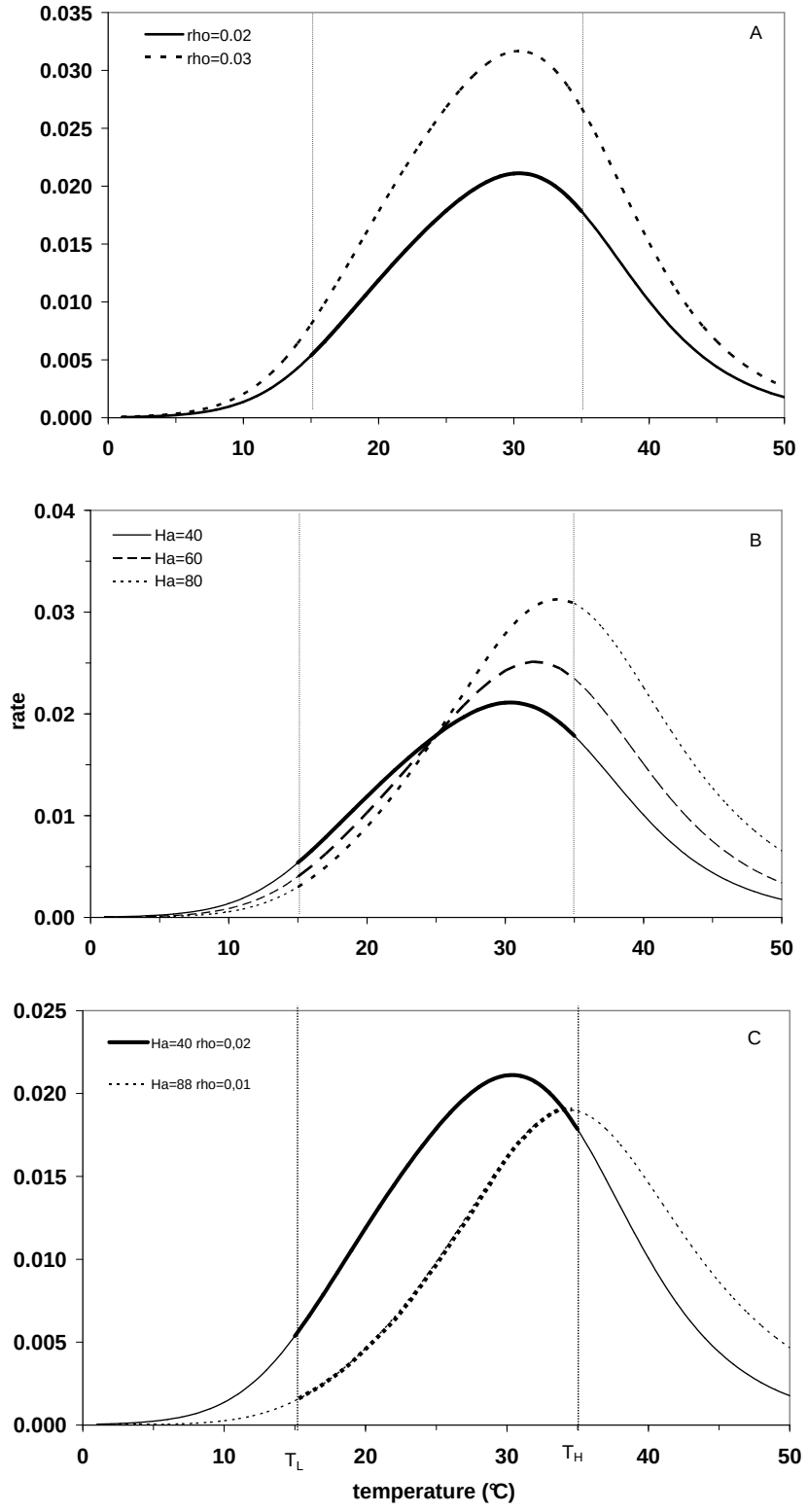

Figure 2. A. The temperature dependence of biological rates according to the Sharpe-Schoolfield equation with A. variation in ρ. The biophysical parameters are set at ρ_{To} = 0.02, H_A=40, H_L=-200, H_H =200, T_L =15°C and T_H =35°C (continuous line), and ρ_{To} =0.03 (broken line). B. variation in H_A: ρ_{To} = 0,02, H_A =40, H_L =-200, H_H =200, T_L =15°C and T_H =35°C (continuous line), and H_A =60 (broken line) and H_A =80 (dotted line). C. variation in both ρ_{To} and H_A: ρ= 0,02, H_A =40, H_L =-300, H_H =300, T_L =15°C and T_H =35°C (continuous line), and ρ_{To} =0.01, H_A =88 (broken line). H_A: , H_H: , H_L: in KJ mol^{-1}.

Sharpe and DeMichele (1977) developed a model describing the temperature-dependence of biological rates and poikilotherm development in particular, such as differentiation rate, cell division rate or growth rate. Their model is based on the Eyring equation, but is derived from Johnson and Lewin (1946), and in its basic form already proposed by Briggs and Haldane (1925). The model is based on the thermodynamic properties of a system acting as a single, hypothetical, developmental enzyme that is rate limiting to development. This rate-controlling enzyme is assumed to be characterized by a constant molecular population which exists either in active form (at normal temperatures) or in reversibly inactive forms (at high or low temperatures). The biophysical model differs in this respect from the 'thermal performance', where only the decrease in reaction rates at higher temperatures is linked with thermal instabilities of enzymes (Hochachka and Somero, 1984; Heinrich, 1977).
The Sharpe - Schoolfield model includes reversible inactivation at both low and high temperature. It was assumed that a control protein could exist in two temperature dependent inactivation states as well as an active state. At high and low temperatures the protein undergoes a conformational transition rendering the protein inactive. The transitions between energy states are unimolecular and completely reversible and no transitions take place between the high and low inactive states directly. For an individual enzyme molecule the cumulative probability of being in the three energy states is therefore equal to one.

From these assumptions an equation can be derived for the probability P_T that the protein is in active state:

$$P_T = \frac{1}{1 + L + H}. \qquad (4)$$

with $L = \exp\left(\frac{H_L}{R}\left(\frac{1}{T_L} - \frac{1}{T}\right)\right)$ and $H = \exp\left(\frac{H_H}{R}\left(\frac{1}{T_H} - \frac{1}{T}\right)\right)$.

where T_L and T_H are the temperatures (°K) at which the protein has equal probability to be active or inactive by low or high temperature inactivation, respectively. H_L and H_H are the change in enthalpy (J mol^{-1}) associated with respectively low or high temperature inactivation of the enzyme (Sharpe and DeMichele 1977, Schoolfield *et al.* 1981). Fig. 2B in Chapter 3 shows the bell-shaped function generated by equation 4, describing the temperature-dependence of protein activity. Activity curves of proteins and enzymes in particular are usually optimum curves with a gradual decline in activity at low or high temperature.

By combining the Eyring equation (1) with reaction rate kinetics, Sharpe & DeMichele (1977) and Schoolfield *et al.* (1981) derived an equation (the Sharpe-Schoolfield equation) for any rate of development under non-limiting substrate conditions:

$$r(T) = \frac{\rho_{T_o} T P_T}{T_o} \exp\left(\frac{H_A}{R}\left(\frac{1}{T_o} - \frac{1}{T}\right)\right) \qquad (5)$$

where $r(T)$ is the mean development rate ($days^{-1}$) at temperature T (°K), P_T is the probability that the rate controlling enzyme is in an active state as defined by equation (4), R is the universal gas constant (8.314 J K^{-1} mol^{-1}) and T_o is the reference temperature in °K.

In the original formulation Schoolfield *et al.* (1981) used 25 °C (298.15°K) as the standard reference temperature and supposed that at the standard reference temperature no

temperature inactivation of enzymes occurred. It can be argued that the temperature at which no temperature inactivation of enzymes occurs (fig 1B) varies between species or populations. The temperature where temperature inactivation is minimal can be considered as the optimal temperature T_o (in °K), at which most metabolic processes function optimally (Ikemoto 2005). The model parameters T_L and T_H were found to be very close to the empirically derived upper and lower thermal limits (T_l and T_h) of developing ectotherms (van der Have 2002). It is assumed that T_o is at the midpoint between empirically derived T_l and T_h.

The Sharpe-Schoolfield equation has considerable advantages over other expressions of biological rate functions (reviewed in Wagner *et al.* 1984b, Kontomidas 2004). It can accurately describe the temperature dependency of a developmental process over the total range of biological activity, including the quasi-linear region at intermediate temperatures and the non-linear regions at high and low temperatures (Fig. 1). Reversible inactivation of enzymes approximately linearizes the exponential rate function expected from the Eyring equation because in most of the thermal range, except perhaps in T_o, $P_T < 1$. It should be noted that the maximum rate ρ_{max} occurs well above the optimum temperature where the rate controlling enzymes are maximally active.

The slope b of the linear part of the Sharpe – Schoolfield equation (eq. 3) is represented by the tangent in T_o:

$$b = P_T \rho_{To} \left(\left(\frac{H_A}{R T_o^2} \right) + \frac{1}{T_o} - P_T \left(\frac{H_L L + H_H H}{R T_o^2} \right) \right) \tag{6}$$

If close to the optimal temperature T_o, no enzyme inactivation, is present, the slope b can be approximated by

$$b = \rho_{T_o} \left(\frac{H_A}{R T_o^2} + \frac{1}{T_o} \right), \tag{7}$$

which is the tangent of the Eyring equation at T_o. Both for the Sharpe-Schoolfield equation and the Eyring equation, the thermal constant for development (in degree-days) equals S = 1/b, where b is the slope of development rate with temperature (in °C) and the threshold temperature (in °C) $h = T_o - \rho_{To}/b$. This threshold temperature can be written as:

$$h = T_o - \frac{P_T \rho_{To}}{b} = T_o - \frac{1}{\left(\left(\frac{H_A}{R T_o^2} \right) + \frac{1}{T_o} - P_T \left(\frac{H_L L + H_H H}{R T_o^2} \right) \right)}. \tag{8}$$

This implies that if only the development rate at the reference temperature, ρ_{To}, varies, and all thermal parameters are kept constant, the slope of the linear part will vary with ρ_{To} but the threshold temperature h will remain constant. This situation applies to developmental rate isomorphy (Jarošik *et al.* 2002, 2004), the observation that in many insect species the temperature sensitivity varies with developmental stages but the threshold temperature h remains constant. We, therefore, propose that the Sharpe – Schoolfield model provides a mechanistic explanation for developmental rate isomorphy.

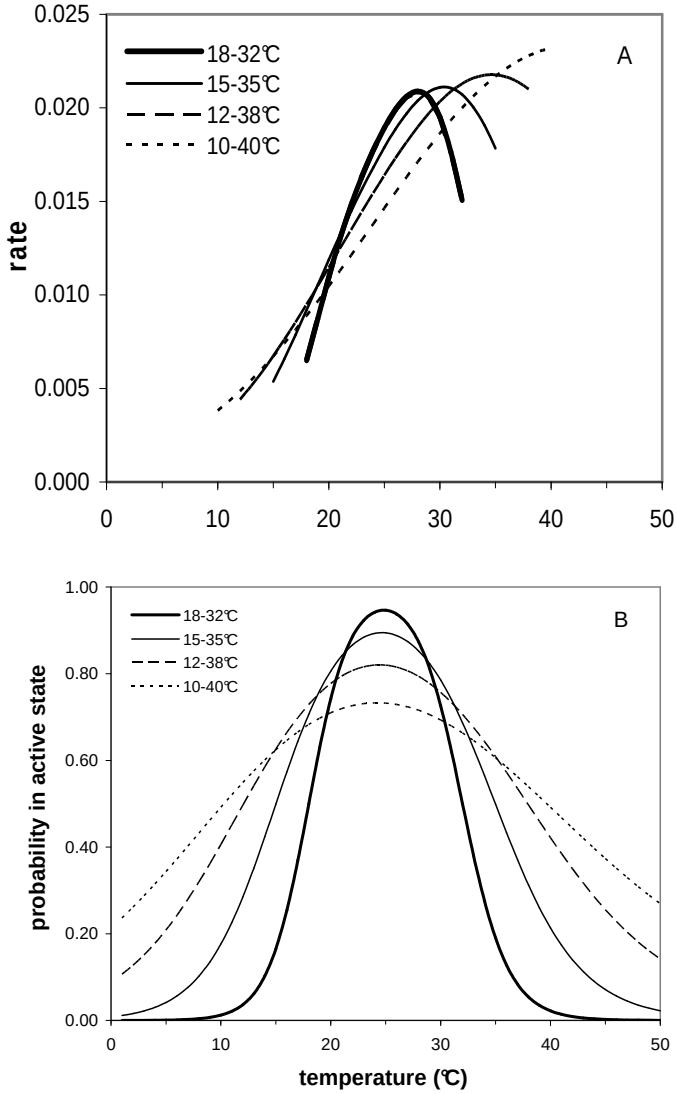

Figure 3. A. The temperature dependence of biological rates according to the Sharpe-Schoolfield equation of organisms adapted to different thermal ranges and identical optimum temperature. B. Temperature dependence of the probability in active state of proteins being adapted to different thermal environments (different thermal ranges, but identical thermal optimum, T_o). The biophysical parameters are set at ρ_{To} = 0,02, H_A =40, T_L =18°C, T_H =32°C, H_L =-360, H_H =360 (thick continous line); T_L =15°C, T_H =35°C, H_L = -200, H_H =200 (thin continuous line); T_L =12°C, T_H =38°C, H_L = -120, H_H =120 (broken line); T_L =20°C, T_H =40°C, H_L =-80, H_H =80 (dotted line). H_A: , H_H: , H_L: in KJ mol^{-1}.

MODEL PREDICTIONS

We assume that all thermal parameters of the Sharpe – Schoolfield model can show heritable variation and are thus subject to selection and evolution (De Jong & Imasheva 2001, De Jong & van der Have 2007). In this section we explore the phenotypic consequences of this variation which could occur within and among populations but also among species. Figure 2A shows that, if only the scaling factor, the reference rate ρ_{To}, varies, the slope of the linear part increases but the extrapolated reaction norms cross at the same threshold temperature *h*. As mentioned above, this variation could be related to the developmental rate isomorphy among different life stages (Jarošik *et al.* 2002, 2004). Among different species this variation could be related to varying genome sizes. A common observation is that development is slower in species with larger genome sizes (Gregory 2001). A different genome size is not expected to have any direct effect on the thermal sensitivity of metabolism. If H_A is varied the slope of the linear part changes and the different reaction norms cross at T_o (Figure 2B). If both ρ and H_A are varied certain combinations could generate parallel reaction norms (Figure 2C). This pattern applies to the vertical shift (faster – slower) in thermal adaptation.

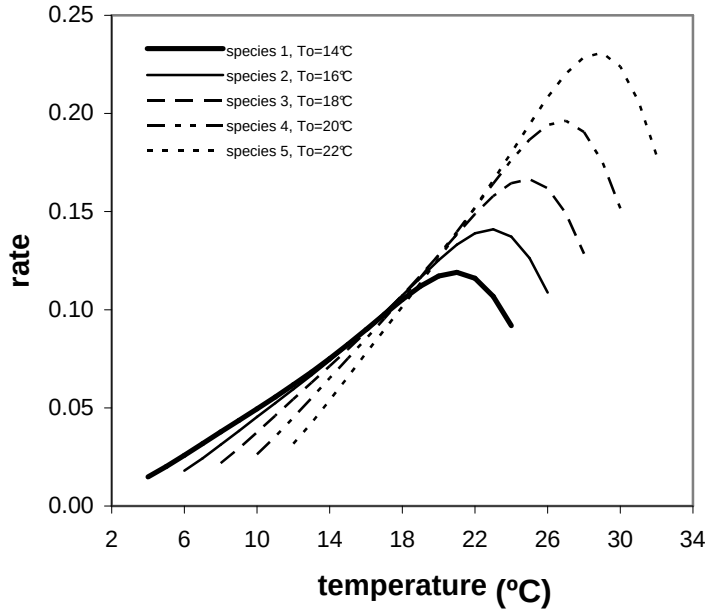

Figure 4. The temperature dependence of rates: between – species variation in thermal limits with thermal range T_r = 20°C. Five species are depicted ranging from a cold adapted (range 4-24 °C) to a warm adapted species (12-32 °C). The optimal temperature for each species is found at the midpoint of its thermal range. The per species reference rate ρ_{To} is taken from an Eyring equation with ρ=0.2 t^{-1} at T_o=25°C. All other parameters are identical between species: H_A =60 KJ mol^{-1}, H_H=400 KJ mol^{-1} and H_L =-300 KJ mol^{-1}.

The change from a specialist to a generalist genotype refers to a change in thermal range T_r, which is changing T_L together with H_L, and T_H together with H_H, and keeping the other parameters including T_o constant in the Sharpe – Schoolfield model. As a result the maximum activity at T_o of the "generalist" genotype is lower than that of the "specialist" genotype (Figure 3) which conforms to the well known trade-off of enzyme stability against enzyme activity (Hochachka & Somero 1983). It also shows that the performance of the specialist is only superior to the jack-of-all-trades close the optimum temperature. Furthermore, the specialist genotype also has a higher thermal sensitivity than the generalist

genotypes without any change in ρ_{To} and H_A and a larger non-linear part of the thermal reaction norm.

A horizontal shift in thermal adaptation (hotter – colder) can be realized by changing both the thermal limits (T_L and T_H) and the optimum temperature T_o, as well, while the thermal range and the other thermal parameters remain constant (Figure 4). This pattern is commonly found in groups of related species. In this case the per species reference rate ρ_{To} is taken from an Eyring equation with ρ=0.2 t^{-1} at T_o=25°C. The slope of the linear part, therefore, increases with temperature approximately as eq. 4. Each species has the highest performance close to its optimum temperature, but overall the difference is smallest around 18 °C, the mean optimum temperature of this group of species. The overall pattern in sensitivity shift is similar to the sensitivity shift resulting from variation in H_A (Figure 2B).

We have shown that all three major patterns in thermal adaptation can be generated by small changes in the biophysical parameters of the Sharpe – Schoolfield model. Sensitivity shift is a fourth major pattern which should be considered in studies of thermal adaptation. A shift in sensitivity can result directly from variation in ρ_{To} or H_A, and indirectly from a shift in thermal range or optimal temperature. Therefore, a phenotypic shift in sensitivity is not informative about the underlying change in thermal parameters included in the Sharpe – Schoolfield model.

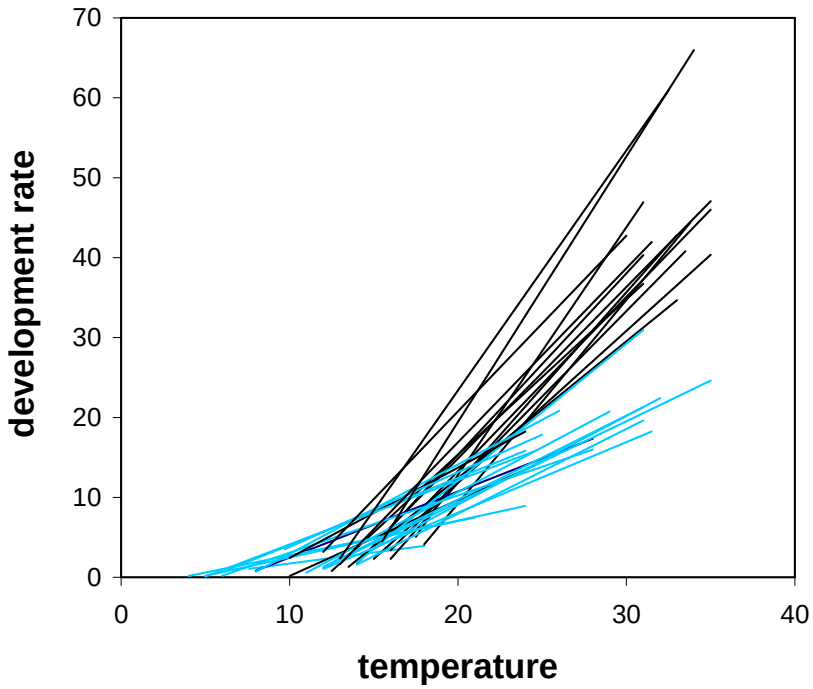

Figure 5. Temperature – development rate reaction norms of 49 anuran species including warm-adapted genera (*Bufo*, *Hyla*, *Microhyla*, *Scaphiopus*, black lines) and cold-adapted genera (*Ascaphus*, *Rana* and *Rana pipiens* sibling species complex, grey lines).

THERMAL REACTION NORMS IN ANURANS

Anurans are particularly well suited to analyze patterns of thermal adaptation because a very complete set of information is available on embryonic development in the literature: developmental rates measured over a wide range of temperatures and experimentally determined thermal limits of development complemented with good data on egg size and

genome size (for references see Appendix 2). From these data slope (*b*) and y-intercept (*a*) of the thermal reaction norm were estimated with ordinary least square regression for each species. An Arrhenius plot, the linear regression of the logarithm of development rate with the inverse temperature $1/T$ (in °K) allows H_A to be estimated from its slope $b_A = -H_A/R$ (eq 1). This situation is equivalent to the two-parameter Sharpe – Schoolfield model and the Eyring model (van Straalen, 1994). For all regressions $R^2 > 0.97$ and for all regressions and regression variables $P < 0.001$ and for most of them $P < 0.0001$. The optimum temperature was calculated as the median of the empirical thermal limits. ρ_{To} was calculated at T_o with *a* and *b* (for the original and derived data see Appendix 2). Literature data were found for nine species groups including six genera mainly from temperate regions (*Ascaphus*, *Bufo*, *Hyla*, *Microhyla*, *Rana* and *Scaphiopus*). A species group was arbitrarily characterized as warm- or cold adapted if the mean optimum temperature was above or below 20 °C, respectively.

Warm-adapted species typically show steeper reaction norms than cold-adapted species (Figure 5) and slope and y-intercept are highly correlated (Figure 6) among all species groups. A "common temperature" (Ikemoto 2005) can be calculated from the slope-intercept relationship within species groups and these "common temperatures" conform very well with the mean optimal temperatures in five species groups with five or more species (Table B.1 in Box 1). The Sharpe – Schoolfield model would predict this pattern if the optimal temperature would change slightly among related species with an accompanying change in slope following the Eyring equation. But a species-specific change in H_A cannot be ruled out.

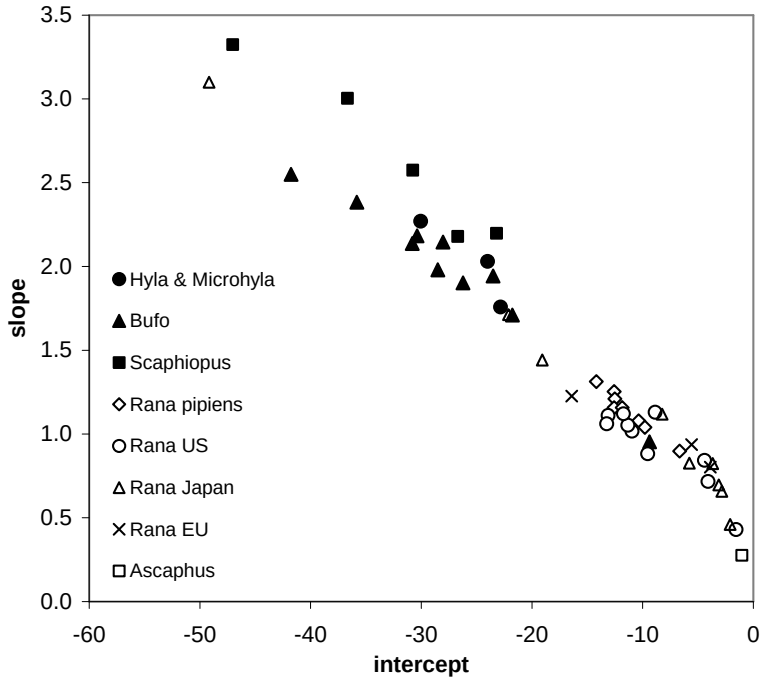

Figure 6. The relationship between slope and intercept of the temperature – development rate reaction norms in 49 anuran species including six genera (*Ascaphus*, *Bufo*, *Hyla*, *Microhyla*, *Rana* and *Scaphiopus*) and the *Rana pipiens* sibling species complex.

The species-specific estimates for H_A derived from Arrhenius plots varied between 38.3 and 114.4 KJ/mol, with a mean of 83.1 (n=49, 95% C.I. 78.8 – 87.4 KJ/mol; range 0.40 – 1.18 eV, mean 0.86 eV and 95% C.I. 0.82 – 0.91 eV). The species-specific development rate ρ_{To} increases exponentially with increasing T_o (Figure 8). The overall pattern can be fitted to

the Eyring equation (eq. 1) with non-linear regression to estimate mean values for H_A, ρ_{To} and T_o and the resulting residuals might have some additional biological meaning. The result is shown in Figure 7A: the regression is highly significant ($R^2 = 0.704$) with H_A = 87.5 KJ/mol (95% C.I. 11 - 108 KJ/mol), T_o = 293.8 °K and ρ_{To} = 14.3. The predicted values for ρ_{To} can be used to predict the overall relationship between slope and optimal temperature with equation 4 (Figure 7B). The species-specific predicted slopes are close to the observed slopes (Figure 8). It should be noted, that although the Eyring model was significantly better than a linear (*d.f.* =1, 45, $P<0.01$), it did equally well compared to a more simple exponential model.

The thermal range was determined in 44 species and varied between 11.4 to 26.5 °C with mean of 20.0 °C. The relationship of T_r with T_o was quadratic ($R^2 = 0.38$, $P < 0.01$) with a maximum at 18.7 °C (Figure 7C). This would suggest that anurans are eurytherms or thermal generalists at intermediate, but more stenotherm or thermal specialists at extreme temperatures. This pattern also occurs within two species groups (*Rana* US, *Rana* Japan).

Although the Eyring equation explains much of the variation in ρ_{To} among species, considerable variation around the predicted line remains. The standardized residuals were tested both against egg size (in mm, or converted to a sphere with specific weight of 1.0) and genome size (pg/2N). Egg size was not correlated with the residuals ($R^2 = 0.04$, *d.f.* = 45, P = 0.17), but highly correlated with genome size ($R^2 = 0.717$, *d.f.*=33, $P < 0.001$; Figure 9). Egg size is also highly correlated with optimal temperature (Figure 10, $R^2 = 0.527$, $P < 0.001$, n = 46) and suggests it is optimised to the thermal environment.

We can conclude that all four major patterns in thermal adaptation (horizontal, vertical, sensitivity (Figure 7A-B) and specialist – generalist shift (Figure 7C) across nearly 50 species of anurans. The Eyring equation explains most (non-adaptive) variation in ρ_{To} (vertical and sensitivity shift, 70,4 %) among species and ρ_{To} explains $_{together}$ with H_A much variation in slope (sensitivity shift). Genome size explains significantly residual (adaptive) variation around the $\rho_{To} - T_o$ relationship. The generalist – specialist shift in thermal range T_r was highly dependent on the species-specific optimal temperature. We propose that 'sensitivity shift' should be considered as a fourth major pattern in thermal adaptation in ectotherms.

DISCUSSION

Our analysis of the patterns in thermal adaptation of development rate in anurans suggests that adaptation to a different T_o (horizontal shift) will lead to the evolution of a different ρ_{To} (vertical shift), a different slope (sensitivity shift) and a different thermal range (generalist – specialist shift) as correlated response. These patterns occurred in all species groups. Development rate ρ_{To} and slope are apparently strongly influenced by the thermodynamic constraint of the Eyring equation, which is also strongly apparent in the Sharpe – Schoolfield model (Figure 4). A decrease in thermal range T_r with increasing temperature could well be explained by the trade-off between thermal stability and catalytic efficiency. Enzymes can compensate for high temperature inactivation by having more weak bonds to stabilize protein conformation but at a cost of a decrease in catalytic efficiency (Hochachka & Somero 1984). Apparently, this compensation also comes at a cost of the thermal range of activity and not only at high but also at low temperatures. When the variation within and among species is considered within a small range of environmental temperatures much residual variation is explained by genome size (in ρ_{To}, Figure 9) and H_A (in slope, Figure 8)). The scale of these effects can also be predicted from the Sharpe – Schoolfield model (Figure 2A-B). Larger genomes slow down in particular embryonic development, because DNA-replication takes

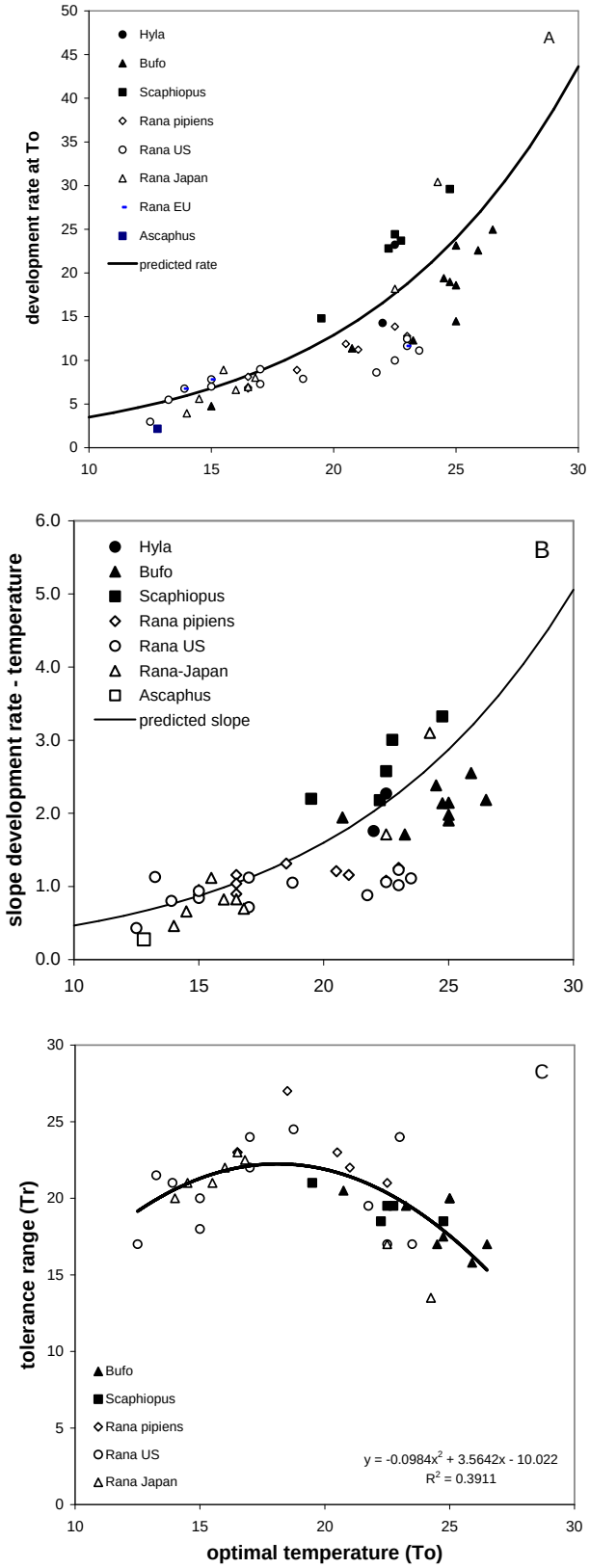

Figure 7. A. Development rates at the optimal temperature (ρ_{To}) increase with optimal temperature T_O in 49 anuran species. B. Slopes of temperature – development rate reaction norms increase with optimal temperature T_O. C. Tolerance ranges are broadest at intermediate optimal temperatures (16-20 °C) and smaller towards lower and higher temperatures.

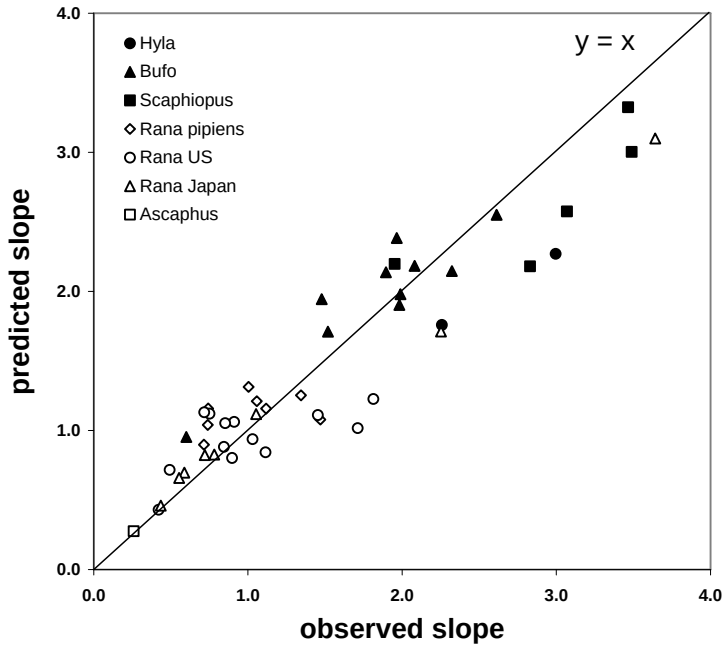

Figure 8. Predicted slope (equation 7) plotted against the observed slope of the temperature – development rate reaction norm in 49 anuran species including six genera (*Ascaphus*, *Bufo*, *Hyla*, *Microhyla*, *Rana* and *Scaphiopus*) and the *Rana pipiens* sibling species complex.

longer (Gregory 2001) and the S-phase takes up to two-third of the total time of the cell cycle (Watanabe & Okada 1967).

The Eyring equation is in its basic form very similar to the equation for temperature dependence proposed by Gillooly *et al.* (2001) (equation 3). Its central role in the Sharpe – Schoolfield equation makes it a good choice for cross-species comparisons, but the linearity of development rate over a large temperature range warns against using the Eyring equation, and the UTD, within species. The Sharpe – Schoolfield model not only predates the UTD model of Gillooly *et al.* (2001, 2002) by several decennia, but also fulfils most of the requirements of the ideal model which can describe the temperature dependence of biological rates within and among species, as well as giving more insights in the proximate mechanisms of thermal adaptation. The thermodynamic parameters explain the linearity of development rate in the large middle part of the reaction norm by the combination of temperature-induced inactivation at low and high temperatures. Interestingly, a strictly linear middle part of the reaction norm of development rate requires that the underlying enzyme is never fully active. Animals should never work all out at 100% capacity. Moreover, the thermodynamic parameters in the Sharpe-Schoolfield model can easily be linked to heritable variation.

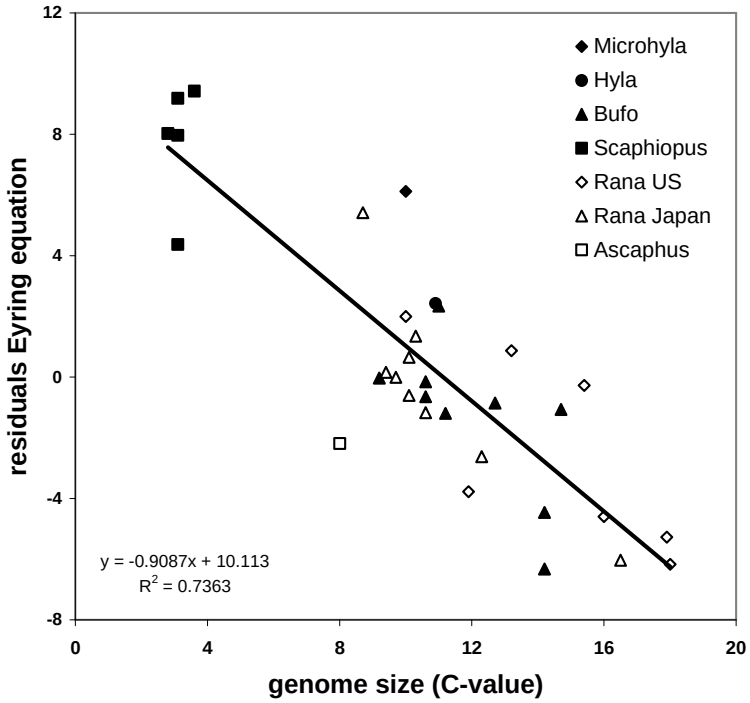

Figure 9. The relationship between the residuals of the nonlinear regression with the Eyring equation and genome size in 37 anuran species including six genera (*Ascaphus*, *Bufo, Hyla, Microhyla*, *Rana* and *Scaphiopus*) and the *Rana pipiens* sibling species complex.

The 95% C.I. (78.8 - 87.4 KJ/mol or 0.82 - 0.91 eV) for the average activation energy of development rate of nearly fifty species of anurans does not include the 'predicted' value of 58 - 68 KJ/mol (0.6 - 0.7 eV) proposed by Gillooly *et al.* (2001, 2002). This value has gained a central role in the UTD but is calculated from activation energies in only two publications (Vetter 1995, Raven & Geider 1988). The average value of 44 activation energies for at least eight different metabolic processes is 53.5 KJ/mol (Raven & Geider 1988, 95% C.I. 45.1 - 61.9 KJ/mol, excluding diffusion processes and temperature insensitive photosynthesis). The average value of 20 activation energies of sets of homologous enzymic reactions reported in Hochachka & Somero (1984) was 64 KJ/mol. It should be noted that these values also depend on the species-specific environmental temperature, cold-adapted species having lower values (30 – 60KJ/mol) than warm-adapted species, which indicates their higher catalytic efficiencies (Hochachka & Somero 1984). The mean activation energy for anuran embryonic development is much closer to the activation energy of cellular processes related to DNA replication, 97.8 KJ/mol (95% C.I. 85.2 – 110.5 KJ/mol, n=6, Cleaver 1967, Spiegler & Norman1970, Watanabe & Okada 1967). This seems to be more in accordance with the fact that embryonic development is much more dominated by cell division than cell growth. For example, no transcription occurs in the first twelve rounds of synchronous cell divisions in *Xenopus* (Newport & Kirschner 1982 a, b). Watanabe & Okada (1967) suggest that the activation energy of the complete cell cycle, which includes DNA-replication in the S-phase and growth in the G2-phase, can be calculated by summation of the weighted activation energies for each part of the cell cycle. As DNA-replication takes up more time of the total cell cycle than growth, the overall activation energy is higher than the unweighted mean.

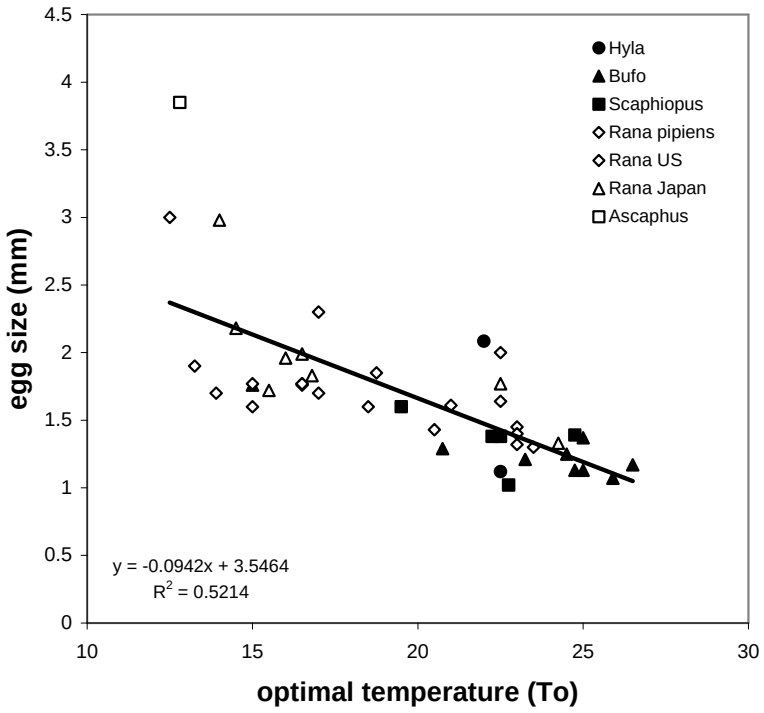

Figure 10. The relationship between egg size (mm) and optimal temperature in 46 anuran species including six genera (*Ascaphus*, *Bufo*, *Hyla*, *Microhyla*, *Rana* and *Scaphiopus*) and the *Rana pipiens* sibling species complex.

Gillooly *et al.* (2002) assume that (1) the hatching larva is larger if it hatches from a larger egg and (2) that a larger egg increases embryonic development time. The second assumption is based on the correlation between egg size and (temperature-corrected) development rate in a wide range of species (Gillooly & Dodson 2000). In marine invertebrates the opposite is found: development time is inversely proportional to egg size with developmental time corrected for temperature (Levitan 2000), which is also confirmed experimentally by manipulating egg size (Sinervo & McEdward 1988). The positive correlations between egg size and development rate found by Gillooly & Dodson (2000) are most likely caused by the high correlation between species-specific egg size and environmental temperature among a wide range of species (insects, Fox & Czesak 2000, marine invertebrates, Levitan, 2000, anura, this paper). The tendency of polar marine invertebrates to have large, yolky eggs is known as Thorson's Rule (Thorson 1950). Correcting for egg size, therefore, removes much of the temperature dependence of development rate en may lead to an underestimate of the temperature sensitivity. The dependence of egg size on environmental temperature is also ignored in other studies of embryonic development time of ectotherms (Hirst & López 2006, Summers *et al.* 2006)

When comparing the development rates of different species it is also important that the developmental stage used to determine the rate is essentially the same. This applies to all the developmental rate data of anurans used in our analysis (stage 20, gill circulation, hatching occurs in stage 25, Gosner 1960). Times to hatching in cross species comparisons are not a clear indication of rates of development (Strathmann *et al.* 2002).

Why is egg size adapted to the thermal environment in a wide range of ectotherms? The strong negative correlation between egg size and environmental temperature may be caused by the significant difference in temperature sensitivity of metabolism (growth) and DNA-replication (differentiation) mentioned above. The high temperature sensitivity of

DNA-replication may limit development at low temperatures much more than growth.

Therefore, it takes more energy to complete development at low temperatures compared to high temperatures and more energy should be stored in the egg for the embryo which cannot actively forage. Summers *et al.* (2007) suggested a similar explanation for altitudinal variation in egg size in anurans. A similar explanation has been put forward by Ernsting & Isaaks (1996) for the (intraspecific) egg size plasticity in relation to the temperature during egg formation.

Our analysis suggests that the sensitivity of development rate of anurans is strongly influenced by thermodynamics. This one group, anurans, can be characterized by a similar Eyring equation, at a similar ρ_E and a similar value of the enthalpy of activation H_A , over the whole group. Adaptation to a different temperature range and a different optimal temperature T_o is the primary process in their thermal evolution. However, there is still much scope to modulate thermal sensitivity through variation in genome size or enzyme efficiency (too reflected by H_A) within thermal environments. In thermal adaptation ectotherms are slaves to the Eyring equation over a range of climates and latitudes, but within the same thermal environment this burden is common to all coexisting ectotherms and many other processes overall thermodynamics become important. Evolutionary interest should be in the change of 'underlying' Eyring equation parameters between groups of animals, the changes along this Eyring equation between species within a group, and the specific adaptations within a species that might be described by the Sharpe-Schoolfield equation.

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Appendix 1. List of parameters.

Name	Interpretation	Units	Values	Source
C-value	Genome size	Pg/2N		
Dvt	Development time	time		
Dvr	Development rate	1/time		
ρ_{To}	Development rate at T_o	1/time		
P	Development rate	1/time		
e_s	Egg size	mm		
e_w	Egg weight	g		
R	Gas constant	J/°K/mol	8.134	
K	Boltzmann's constant	eV	8.02	
T	Temperature	°K		
T_o	Optimal temperature	°C or °K		This paper/thesis
T_l	Lower thermal limit (empirical)	°C		This paper/thesis
T_h	Upper thermal limit (empirical)	°C		This paper/thesis
T_r	Thermal range	°C	$T_l+(T_h-T_l)/2$	This paper/thesis
T_{sp}	Spawning temperature	°C		This paper/thesis
r (T)	Development rate	1/time		Schoolfield *et al.* 1982
H_A	enthalpy of activation	J/mol		Schoolfield *et al.* 1982
T_L	Temperature at which the enzyme has equal probability to be active or inactive by low temperature inactivation	°K		Schoolfield *et al.* 1982
T_H	Temperature at which the enzyme has equal probability to be active or inactive by high temperature inactivation	°K		Schoolfield *et al.* 1982
H_L	Enthalpy of inactivation at low temperature	J/mol		Schoolfield *et al.* 1982
H_H	Enthalpy of inactivation at high temperature	J/mol		Schoolfield *et al.* 1982
P_T	Probability that enzyme is in active state			Schoolfield *et al.* 1982
H	Threshold temperature for development	°C		Trudgill *et al.* 2005
A	Intercept of temperature - development rate reaction norm	°C		This paper/thesis
B	slope of temperature - development rate reaction norm	rate/°C		This paper/thesis
S	Thermal time (in degreedays)	°C*time		Trudgill *et al.* 2005
A	Temperature dependence			Charnov & Gillooly 2003
E	Average activation energy of metabolic reactions	eV	0.6 - 0.7	Gillooly *et al.* 2002
T_{zero}	Freezing point of water	°K	273	Gillooly *et al.* 2002
T_c	Developmental temperature	°C		Gillooly *et al.* 2002

BOX 1. ADAPTATION TO ENVIRONMENTAL TEMPERATURE IN ANURANS

T.M. van der Have

If the (empirical) optimal temperature derived from the thermal limits is adapted to the environmental temperature where a species occurs it is expected that the optimal temperature is lower at higher latitudes where the climate is colder. Furthermore, also the spawning temperature in spring should closely match the optimum temperature of development. Both predictions were tested with literature data (see references below), which included the locations of the sampling sites and the temperature during spawning.

Regression of optimal temperature versus latitude (Figure B.1A) shows that species that occur (and were sampled) at higher latitudes have lower optimum temperatures than species that occur at lower latitudes. It should be noted that the variation among species at the same latitude is considerable. Regression of spawning temperature versus optimal temperature (Figure B.1B) shows that the optimal temperature is highly correlated with optimum temperature, but that frogs and toads spawn at a temperature which is several degrees lower that the optimal temperature T_o for development. This suggests that with increasing spring temperatures, the environmental temperatures during the development of later larval stages closely match their optimal temperatures. Both correlations suggest that the optimal temperature derived from the empirical thermal limits is a good indication of adaptation to the environmental temperature regime.

Table B.1. Range and mean of optimal temperatures (T_o) and calculated "common temperature" with sample size (N) of nine species groups of frogs and toads including six genera (*Ascaphus*, *Bufo*, *Hyla*, *Microhyla*, *Rana* and *Scaphiopus*) and the *Rana pipiens* species complex.

	optimal temperature (T_o)					
species group	mean	range	N	"common temperature"	N	thermal adaptation
Microhyla	27.0		1			warm
Hyla	24.6	22.9 - 23.5	2			warm
Bufo (excl. *B. bufo*)	24.5	20.8 - 26.5	9	27.8	9	warm
Scaphiopus	22.3	19.5 - 24.8	4	21.4	5	warm
Rana pipiens complex	19.4	16.5 - 23.0	8	19.5	8	cold/warm
Rana US	18.4	12.5 - 23.5	10	19.7	9	cold/warm
Rana Japan	17.4	13.9 - 24.3	5	18.7	10	cold/warm
Rana Europe	14.5	13.9 - 15.0	3			cold
Ascaphus	11.5		1			cold

A “common temperature” (Ikemoto 2005) can be calculated from the slope-intercept relationship within species groups and these “common temperatures” conform very well with the mean optimal temperatures in five species groups with five or more species (Table B.1 in Box 1). This pattern confirms the broad distinction between warm-adapted species (tree frogs *Hyla* and *Microhyla*, and toads, *Bufo* and *Scaphiopus* and the more cold-adapted frogs *Rana*. The Tailed Frog (*Ascaphus truei*) seems to be a real cold specialist (see also Figure 8C).

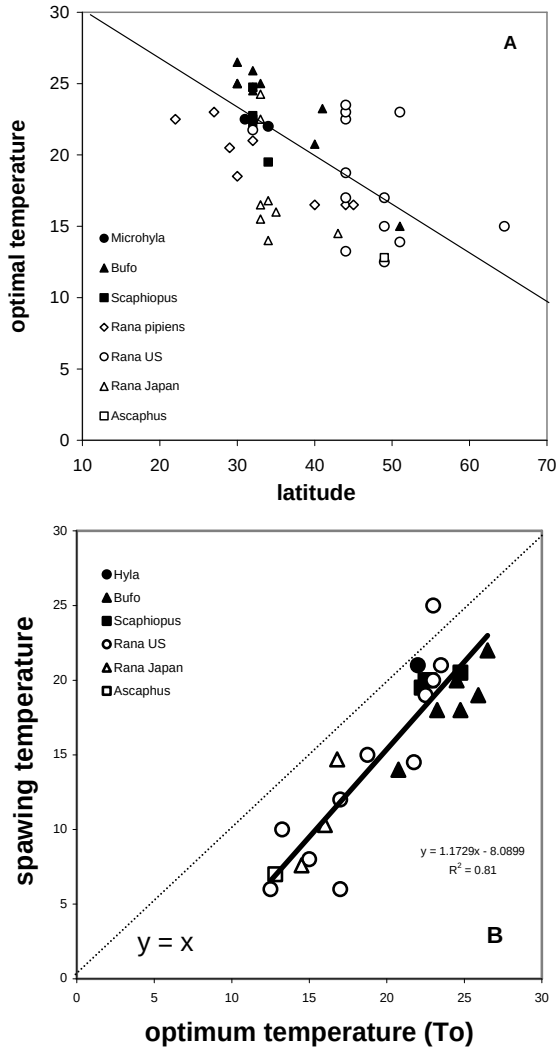

Figure B.1. The relationship between optimal temperature (T_o) and (A) latitude and (B) spawning temperature in, respectively, 49 and 26 anuran species including six genera (*Ascaphus*, *Bufo*, *Hyla*, *Microhyla*, *Rana* and *Scaphiopus*) and the *Rana pipiens* sibling species complex.

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CHAPTER 6

SYNTHESIS: ARE ECTOTHERMS SLAVES TO THE EYRING EQUATION?

T.M. van der Have

Introduction

The main aim of this thesis was to establish to what extent the thermodynamics of biological rates constrains the thermal adaptation of developing ectotherms. Thermodynamics are often seen as a constraint to the evolution of metabolic rates (Gillooly *et al.* 2001), development rate (Gillooly *et al.* 2002) and population growth rates (Frazier, Huey & Berrigan 2006). These authors suggest that biochemical adaptation seems to be unable to overcome the "tyranny" of thermodynamics for these biological rates. In other words, although physiological adaptation to, for example, lower temperature allows organisms to invade cold environments, it is seemingly incapable of compensating for reduced rates of metabolism, development or population growth. I approached this problem at two levels, within and among species, and explored the suitability of the biophysical Sharpe – Schoolfield equation to study and explain patterns in thermal adaptation at both levels. During this exercise I focused on four research questions:

1. Why do most ectotherms become smaller when growing faster at higher temperatures and larger when growing slower at lower temperatures?
2. Why are temperature limits in developing ectotherms usually steep and well defined at both low and high temperatures?
3. How can one predict patterns in thermal adaptation within and among species from the kinetics of reaction rates?
4. Are linear temperature-development rate reaction norms approximations of the general temperature dependence predicted by the Eyring equation?

A general conclusion, which transpires from all chapters, is that the biophysical Sharpe – Schoolfield equation is indeed a highly suitable model to study thermal adaptation in ectotherms. In Chapter 2 the equation is applied to growth and differentiation rate separately to model the temperature dependence of size at maturity. In Chapter 3 the reversible inactivation part of the Sharpe – Schoolfield equation (the denominator) is applied to a genetic control system of the cell cycle to model the effect of high and low temperature inactivation. In Chapters 4 and 5 the Eyring part of the Sharpe – Schoolfield equation (the numerator) is used in cross-species comparisons.

In this chapter I will review the general aim of this thesis to explore the thermodynamic constraints in thermal adaptation. First, I take a look at the progress in solving the life-history puzzle of the TSR since the publication of the Van der Have – De Jong model in 1996. Then, I compare the general model for thermal tolerance for developing ectotherms (van der Have 2002) with the oxygen limitation hypothesis for thermal limits in adult ectotherms (Pörtner *et al.* 2007). I continue by discussing the genetics of plasticity, the tradeoffs at the enzyme level and their effects on reaction norms and explore the implications of within-species processes to species interactions. Finally, I discuss the importance of phenotypic plasticity in understanding of evolution.

Temperature - size rule: one model to rule them all?

The observation that ectotherms usually mature at a smaller size at high and at a large size at low growth temperatures despite growing and developing faster at higher temperatures has been known at least since the middle of the last century (Ray 1960). However, it took more time to realize that this widespread phenomenon contradicts classical theories of life-history evolution, which predict a smaller size at maturity in environments that cause growth to proceed slower (Berrigan & Charnov 1994). These authors introduced the term life-history puzzle for this apparent contradiction. At the same time Atkinson (1994, 1995) reviewed the literature and found that in 83% of 109 studies larger sizes were found at cooler temperatures, including protists, plants, a bacterium and many animal taxa. This widespread phenotypically plastic response of body size to growth temperature was subsequently termed the "Temperature – size rule" (TSR, Atkinson & Sibly 1997) and which was the focus of much research in the last decade. Both proximate (Ernsting 1995, Van der Have & De Jong 1996; Van Voorhies 1996) and ultimate explanations (Sibly & Atkinson 1995, Atkinson & Sibly 1996, Partridge *et al.* 1994, Perrin 1995) have been put forward which were reviewed in Atkinson & Sibly (1997). Two publications, in particular, Van Voorhies (1996) and Van der Have & de Jong (1996) sparked considerable controversy because they suggested that temperature-dependent body size variation could be a side-effect of selection on either growth rate, development rate or both, whereas body size itself is usually and intuitively seen as the focus of selection. These proximate and ultimate explanations were subsequently debated, rejected and revisited (Angilletta & Sears 2004). In general, no adaptive explanation was found to be general enough for all ectotherms and mechanistic explanations lacked general empirical support. Several alternatives were proposed combining both adaptive and mechanistic explanations (Angilletta *et al.* 2004, Blanckenhorn & Demont 2004, Kozlowski 2004). To illustrate the major developments in TSR research in the last decade I will review the 67 papers that have cited Van der Have & de Jong (1996) to date.

Our paper included seven different topics referring to either observations, assumptions, conclusions or implications of the model: (1) the observation of TSR in ectotherms; (2) Development is the result of growth and differentiation (3) the sensitivity growth (perhaps protein synthesis) is lower that sensitivity differentiation (perhaps DNA-replication); (4) mechanisms growth and differentiation are fundamentally different, which might impose a constraint; (5) physiological time; (6) egg size also follows the TSR; (7) cell size varies as a result of interaction between growth and differentiation. The following papers studied different aspects of the thermal biology of a range of organisms, but were not aimed to test the different TSR hypotheses. The TSR was observed in house crickets (with different temperature sensitivities of growth and differentiation, Booth & Kiddell 2007), two species of weevil (altitudinal growth rate clines related to gradients in temperature and length of growth season, Chown & Klok 2001, 2002), a drosophilid (David, Legout & Moreteau 2006, the overall relationship was non-linear, body size decreased near the lower thermal limit) an introduced fish (Dembski *et al.* 2006), an introduced drosophilid (clinal variation in body size and wing loading, Gilchrist & Huey 2004), Australian frogs (Schäuble 2004), a caddisfly (seasonal variation in body size, Spänhoff 2005). Two papers studied growth rate (nine species of marine fish, Neuheimer & Taggart 2007, heritability of growth rate thermal reaction norm (collembolan insect,

Driessen, Ellers & van Straalen 2006), distribution and performance of two temperate species of frog (Halverson *et al.* 2003), climatic effects on caterpillar fluctuations (Reynolds *et al.* 2006) and the effect of temperature on pupal development time and morphology in a butterfly (Stevens 2004). The latter study found differential effects of temperature on different body parts during metamorphosis, when no growth occurs. Two studies found a converse TSR in sperm size of dung flies (Blanckenhorn & Hellriegel 2002) and in a body size cline of a grasshopper (Berner & Blanckenhorn 2006).

Recent studies of latitudinal body size clines suggested an adaptive explanation for shell morphology of a marine gastropod (a latitudinal gradient in predation pressure: Irie & Iwasa 2005) and a combination of proximate and ultimate explanations for geographical variation in body size of a drosophilid (the accessibility of glycogen storage as the proximate mechanism responsible for the life-history tradeoff between larval survival and adult size: Bochdanovits & de Jong 2003) and dwarfism in a marine fish (temperature and resource effects as proximate and ultimate explanations, respectively, Sonin *et al.* 2007). In another study (latitudinal wing length clines at three continents in a drosophilid: Gilchrist *et al.* 2004) the exact adaptive significance of increased wing size at higher latitudes remained unanswered.

Only three studies that cited our paper dealt with the importance of physiological time (topic 5), when comparing the temperature dependence of growth and development rate: a study of the temperature dependence of growth rate under varying resource levels in three fly species (Blanckenhorn 1999), growth rate of nine species of marine fish (Neuheimer & Taggart 2007) and the temperature dependence of embryonic and larval development time in a collembolan insect species (Stam 1997).

Several papers questioned the existence of physiological constraints based on empirical evidence and presented alternative, adaptive, explanations for the TSR in body size (topic 1, Atkinson, Morley & Hughes 2006, Kingsolver, Massie & Smith 2007) and egg size (topic 6, Fischer, Brakefield & Zwaan 2003, Fischer, Bauerfeind & Fiedler 2006, Steigenga *et al.* 2005) or found evidence for the absence of an effect on cell size (topic 7, Litzgus, DuRant & Mousseau 2004). Atkinson, Morley & Hughes (2006) proposed a unifying adaptive hypothesis that predicts how temperature affects the sizes of ectothermic mitochondria, cells, organs, modules and organisms, and their relationships with processes that determine the functional capacity of aerobic metabolism. Low temperatures inhibit rates of oxygen consumption, but oxygen transport from the external environment to the mitochondria relies on diffusion, which is relatively temperature insensitive. At high temperatures the rate of oxygen consumption increases, which leads to greater difficulty in meeting tissue oxygen demands if gas exchange systems include a diffusional step (Pörtner 2001, 2002). A general reduction of cell size with increasing temperature will help to improve oxygen supply to mitochondria within cells by reducing the distance to the cell surface. This hypothesis seems to be supported by the patterns in cell size and cell number responses at various systemic levels (cells, organs, modules in autozooids) to different temperatures and oxygen levels. Their conceptual model, however, does not provide an explanation for the exceptions to the TSR or converse Bergmann clines and generally lacks quantitative predictions.

Kingsolver, Massie & Smith (2007) found that two butterfly populations that diverged 150 years ago showed an opposite response of body size to temperature, an increase with temperature in one and a stable or decrease in body size in the other

population. They concluded that rapid evolutionary divergence argues against simple, general mechanistic constraints as the underlying cause of the TSR. Their study included only two experimental temperature regimes. These patterns could still be well explained with the Van der Have – de Jong model if the two populations adapted to a different temperature optimum and/or different thermal limits as body size often stabilizes or decreases close to the lower thermal limit (see also David, Legout & Moreteau 2006).

The paper of Litzgus, DuRant & Mousseau (2004) seems to be an exception, as many studies including a wide range of taxa show that cell size is larger when ectotherms grow at lower temperatures (reviewed by Arendt 2007). Resource limitation usually result in smaller cell numbers and if temperature interacts with resource levels or availability this may lead to a combination of different cell sizes and cell numbers in the field or laboratory (Arendt 2007).

Egg size generally follows the TSR in a wide range of taxa (*e.g.*, arthropods, Fox & Csezak 2000). Several studies of egg size plasticity in a butterfly suggested that the increase of egg size at lower temperatures during egg laying could be adaptive in ectotherms. Fischer, Brakefield & Zwaan (2003) found that selection might favour larger eggs at a lower temperature. Steigenga *et al.* (2005) and Fischer, Bauerfeind & Fiedler (2006) found in experiments with half-sib breeding design and selection lines, respectively, significant genotype by environment interaction for egg size and temperature, which is an important condition for evolutionary change. All three papers suggested that these findings strengthen the support for the adaptive nature of temperature-mediated plasticity in egg size. However, these results do not contradict the Van der Have – de Jong model, which also includes the possibility of genotype by environment variation in growth rate, differentiation rate or body size. As argued in Chapter 5, optimal egg size might be larger (with more energy stores) at lower temperatures because the total amount of energy needed for completing embryonic development is larger compared to high temperatures as a low temperature slows down differentiation (cell division) more than growth (metabolism). This differential effect of temperature cannot be compensated by increased energy acquisition, as eggs usually do not feed.

Theoretical models can be tested if the assumptions or predictions are tested in well-designed laboratory or field experiments. A number of papers fell in this category as most of them first confirmed the existence of the TSR, acknowledged that development is the result of growth and differentiation and tested if growth rate had lower temperature sensitivity than differentiation rate (topics 1-3) or studied variation in egg or cell size (topics 6 - 7). Invariably, the outcome was positive on all most topics. Blanckenhorn & Llaurens (2005) showed that in a dung fly the TSR held for wing cell size and ommatidia size and stated, “The physiological constraint hypothesis remains viable as a proximate, non-adaptive explanation for the TSR in ectotherms”. However, they did not measure selection on any of the relevant life-history characters, and hence cannot conclude that the observed pattern was non-adaptive. Blanckenhorn & Henseler (2005) investigated temperature-dependent ovariole and testis maturation in a dung fly and related it to corresponding temperature effects on pre-adult development time. In accordance with the TSR warmer temperatures resulted in smaller ovarioles (eggs) and smaller testes, independent of body size. Davidowitz, D’Amico & Nijhout (2004) studied how phenotypic plasticity of body size of a hawk moth in response to variation in temperature

is affected by the plasticity in growth rate and two components of development rate which determine the duration of the growth period: the onset of juvenile hormone decay and the timing of ecdysteroid secretion. They concluded that the plasticity of size in response to temperature is regulated by the differential temperature sensitivity of growth rate and the interval to cessation of growth. They generalized this conclusion in other papers (Davidowitz & Nijhout 2004, Nijhout 2003) and argued that life history evolution along altitudinal, latitudinal and seasonal (thermal) gradients may occur through differential selection on growth rate and the duration of two independently controlled determinants of the length of the growth period. This hypothesis can be considered as a combination of proximate and ultimate mechanisms. Dhillon & Fox (2004) studied growth rate and age and length at maturity at different growth temperatures and showed that growth rates increased, but age and length at maturity decreased at higher growth temperatures, except near the upper thermal limit, where this effect was reversed. This unexpected reversal may have been caused by the disturbance of normal gonadal development. Ernsting & Isaaks (2000) explained the increase in egg size with decreasing temperatures by discerning two stages in the process of egg production – follicle production in the germarium (differentiation) and yolk accumulation (growth) in the vitellarium (Nijhout 1994). If the yolk accumulation would be less sensitive to temperature than follicle production egg size would increase with decreasing temperature. Their experiments supported this mechanistic explanation, which is analogous to the Van der Have – de Jong model. Other studies which measured the temperature sensitivity of age and size at maturity (in a butterfly, Fischer & Fiedler 2002, in a tropical and subtropical squid, Forsythe *et al.* 2001, in a seed beetle, Stillwell & Fox 2005), or the temperature sensitivity of growth and development (in an isopod, Helden & Hassall 1998) generally supported the predictions of the Van der Have & de Jong model. Several authors acknowledged the notion that development to maturity can be thought of as having two components – differentiation and growth (Jarošik *et al.* 2004, Trudgill, Honek & van Straalen 2005, Stillwell & Fox 2005).

Most of the papers discussed so far studied either TSR or exceptions to TSR in ectotherms, although the biophysical Van der Have – de Jong model quantitatively can predict both depending on the relative sensitivities of growth and differentiation.

Blanckenhorn & Demont (2004) also proposed that Bergmann and converse Bergmann latitudinal clines in arthropods are two ends of a continuum, which was based on a study of a dung fly and a literature review. Fischer & Fiedler (2002) reached the same conclusion in a study of alpine and lowland populations of a butterfly. Both papers suggested that the interaction between generation time (*e.g.*, uni- or multivoltine) and growing season length can explain the adaptive significance of both TSR and converse TSR. Accordingly, multivoltine species with short generation times benefit from reproducing early at high temperatures, indicating the potential for extra generations, even at the expense of being smaller. Univoltine species should be selected for large body size to maximize adult fitness, and therefore adult size should respond only weakly to temperature. Chown & Gaston (1999) and by Chown & Klok (2002) suggested this earlier. This conceptual model, the generation time – growing season length interaction (GGI) hypothesis, is a mix of proximate and ultimate explanations and may provide a synthesis to the controversy about TSR for body size plasticity and Bergmann's rule for clines (Blanckenhorn & Demont (2004). Other papers, which proposed a combination of

proximate and ultimate explanations for TSR based on theoretical models, are an optimal resource allocation model (Kozłowski, Czarnołeski & Dańko 2004) and an energy-partitioning model including temperature effects on senescence (Kindlmann, Dixon & Dostálková 2001). These models, however, are not at variance with the Van der Have – de Jong model for TSR because they include the possibility of differential sensitivity of growth and differentiation.

A recent paper (Walters & Hassall 2006) takes these ideas much further by building a life-history model with a very complete life-history dataset of a temperate grasshopper showing an exception to TSR (although they do not cite Blanckenhorn & Demont 2004, Chown & Gaston 1999, Chown & Klok 2002, Fischer & Fielder 2002). They showed that it is (ultimately) optimal for this species to mature at a larger size at higher temperatures. Walters & Hassall also showed that plasticity in adult size is (proximately) determined by the relative difference between the minimum temperature threshold for growth and development rates. Walters & Hassall relate this mechanism to the biophysical Van der Have – de Jong model and suggest that ectotherms that obey TSR are identified as having a higher temperature threshold for development rate than for growth rate. Exceptions to the TSR are identified as having a lower temperature threshold for development rate than for growth rate. The latter scenario may arise broadly in two ways. The first adaptive explanation is similar to the GGI-hypothesis of Blanckenhorn & Demont (2004) and Fischer & Fiedler (2002). The second, but not mutually exclusive, adaptive explanation for why the difference between the thermal threshold temperature of growth is higher than that of development is if there is selection for greater thermal specialization or stenothermy in growth rate. Stenothermal species have narrower response curves and exhibit higher plasticity relative to eurythermal species and hence have higher temperature thresholds relative to thermal generalists for a given thermal optimum (Figure 1, see also Figure 3 in Chapter 5). At temperatures close to the thermal maximum for growth rate, stenotherms are expected to have relatively greater fitness than generalists because they can attain a larger and potentially more fecund adult size within a given amount of time. This prediction of Walters & Hassall (2006) that selection of stenothermal growth strategies leads to greater adult size plasticity was confirmed by comparing two stenothermal with two eurythermal grasshopper species. The stenothermal species had the greatest level of growth rate plasticity, the greatest absolute differences in the temperature thresholds for growth (TT_G) and development (TT_D) rate and highest plasticity in adult size compared to two more eurythermal species. Walters & Hassall conclude that whatever the adaptive explanations for the relative positions of the minimum temperature thresholds for growth and development rates, all ectotherms should theoretically conform to an underlying mechanism based on their enzyme kinetics. For a general explanation of the TSR to be truly general, it must include the causal mechanisms that explain why most ectothermic organisms should obey the TSR, as well as why some organisms are exceptions to it (Angiletta & Dunham 2003). Walters & Hassall believe that the mechanistic explanation originally proposed by the Van der Have – de Jong model meets this condition and the authors showed that adaptive explanations for ectotherm responses might be sought in relation to selection pressures

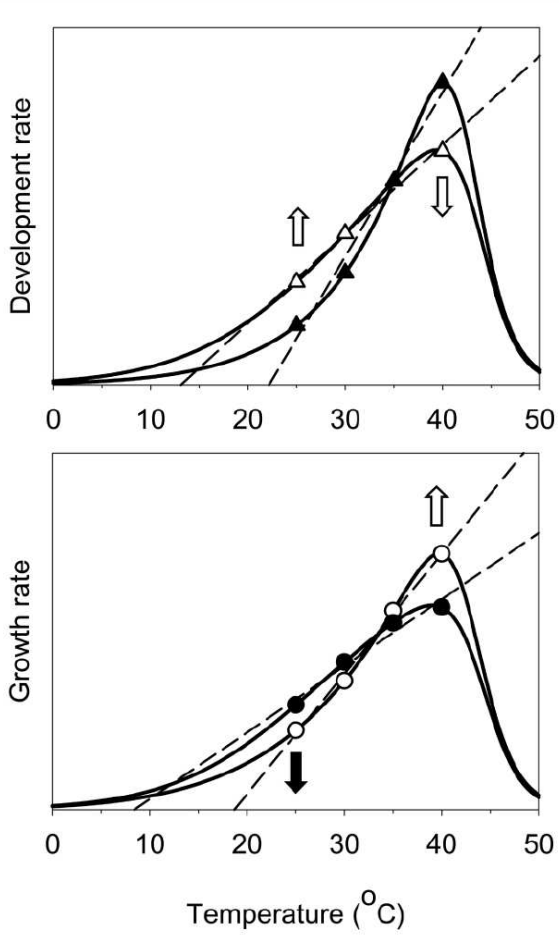

Figure 1. From Walters & Hassall (2006): Two alternative theoretical scenarios depicting how a value of TTG - $TT_D > 0$ might evolve. Solid lines represent empirical rates predicted by the Sharpe-Schoolfield equation; dashed lines represent linear approximations of the quasi-linear region of this relationship over the ecologically relevant temperature range that are used to determine minimum temperature thresholds. Top, lower TT_D might evolve as a consequence of selection for optimal adult size across a range of temperatures (adaptive plasticity). Here greater selection pressure for a lower TT_D is depicted for individuals within a univoltine population (open triangles) relative to a multivoltine population (filled triangles). Bottom, higher TT_G may evolve as a consequence of selection for greater thermal specialization in growth rate. Her greater selection pressure for a higher TT_G is depicted for individuals within a stenothermal population (open circles) relative to a eurythermal population (filled circles). Open arrows indicate direction of selective pressure; filled arrow indicates effects of genetic constraints.

acting on growth and development rates independently. In this respect, ectotherms are not simply slaves to the Eyring equation, but servants, which conform to the thermodynamics underlying enzyme kinetics when seeking evolutionary solutions to environmental problems.

Tolerance limits

Van der Have (2002, Chapter 3) makes the observations that (1) there is an ontogenetic shift in thermal tolerance: the thermal range of viable embryonic development is often much smaller that the thermal tolerance range of adults (at least in insects, fish and amphibians) and (2) thermal limits have a threshold character. A theoretical model is proposed showing that (3) inactivation of proteins and enzymes mimics the dosage change of regulatory components during the cell cycle and (4) the predicted thermal limits based on a proximate model are close to the observed thermal limits in nearly twenty insect species. That paper further discusses the importance of (5) the maximal performance – thermal range tradeoff commonly observed in enzymes and (6) that this may explain the effect of behavioural or physiological fever on pathogens.

Understanding the evolution of thermal tolerance in ectotherms has become an important theme in evolutionary and ecological physiology (Huey & Kingsolver 1993, Angilletta *et al.* 2002. Ontogenetic shifts in thermal tolerance are important because they may be related to ontogenetic shifts in thermal habitats and the thermoregulatory options available for the different life-history stages (Winne & Keck 2005). Ontogenetic shifts are often ignored in studies of thermal tolerance. Castañeda *et al.* (2004) showed that in a comparative study of thermal physiology of three populations of a terrestrial isopod the lower tolerance limit varied among populations while the upper thermal limit did not. This agrees with the global review of 250 insect species from ten orders by Addo-Bediako, Chown & Gaston (2000) that the upper thermal limits of adult viability show little geographic variation. In contrast, the lower lethal limits do decline with latitude, which leads to the broadening of physiological tolerances with latitude. At high latitudes in both Hemispheres the upper thermal limits tend to decline slightly leading to smaller thermal ranges. This pattern could have been more pronounced if only life history stages were included that are most sensitive to temperature extremes. For example, the general pattern found by Addo-Bediako, Chown & Gaston (2000) conforms very well with the trend observed in the thermal range of viable embryonic development in anurans which tend to be smaller (more stenothermic) in species living in low and high temperature environments compared to wider thermal ranges (more eurythermic) in temperate environments (Figure 8C, Chapter 5). Ontogenetic shifts are even more important for pathogens of endotherms with free-living stages or with intermediate, ectotherm host (Holmstad, Hudson & Skorping 2005, Gosh & Bhattacharyya 2007). This implies that there is selection for a wide thermal tolerance range outside the endothermic host, but selection for high performance when entering a host. The outcome of these selection pressures is likely to be constrained by the stability – activity tradeoff in enzymes (Clarke 2003, Fields 2001). This tradeoff might be exploited by infected hosts which increase their body temperature (behavioural fever in ectotherms, physiological fever in endotherms as argued in Chapter 3) or lower their body temperature (Müller & Schmid-Hempel 1993) in response to a pathogen or parasite. The trade-off may explain why

endotherms have a high body temperature, higher than most average habitat temperatures. Pathogens and parasites usually depend on ectotherm vectors to complete their life cycle and therefore have to adapt to both habitat temperature and the higher endothermic body temperature. If body temperature is relatively high compared to habitat temperature then parasites and pathogens have difficulty in maintaining a broad thermal range and high enzyme activity (maximal activity and stability tradeoff). Fever might also be more effective at high temperatures compared to environmental temperatures. This idea is not mutually exclusive with the foraging activity and predator escape hypothesis for the high body temperature of endotherms as proposed by Heinrich (1977).

Knies *et al.* (2006), who investigated the genetic basis of thermal reaction norm evolution in bacteriophage populations, suggested that this tradeoff could be exploited for successful vaccine development strategies. They found that changes in optimal temperature accounted for almost half of the evolutionary change in thermal reaction norm shape and made the largest contribution toward adaptation at high temperatures. They speculate that, if viruses would be artificially adapted to low temperatures this would reduce their virulence at the higher body temperatures of endotherms.

Another hypothesis for thermal tolerance has been put forward which suggests that a mismatch between the demand for oxygen and the capacity of oxygen supply to tissues is a primary mechanism to restrict whole-animal tolerance to thermal extremes (Pörtner 2001, 2002, Pörtner & Knust 2007). In aquatic animals, a gradual decrease in the capacity to perform aerobically characterizes the onset of thermal limitation at both ends of the thermal range. The reduction in aerobic scope is caused by limited capacity of circulatory and ventilatory systems to match oxygen demand. The authors showed that in a temperate fish species thermally limited oxygen delivery closely matches environmental temperatures beyond which growth performance and abundance decrease. This hypothesis is apparently not mutually exclusive with the proximate model presented in Chapter 3, because it seems to be primarily applicable to adult, aquatic organisms (although Atkinson, Morley & Hughes 2006 applied this hypothesis to TSR in all ectotherms). In many insects, fish and amphibians embryonic thermal tolerance ranges are much smaller and the thermal limits steeper than the gradual decrease in aerobic scope of aquatic organisms. However, the gradual decrease in aerobic scope could well be the result of gradual temperature inactivation of metabolic enzymes (Sharpe & DeMichele 1977). The thermal growth reaction norm presented by Pörtner & Knust (2007) shows a linear part and a decline at high temperatures. If enzyme activity of metabolic enzymes was maximal throughout much of the thermal range, then the increase of growth rate would have been exponential and following the Eyring equation (see Chapter 4). Evidently, both hypotheses could be tested in a detailed study of thermal reaction norms of embryonic development and at higher systemic levels.

Two types of thermodynamic constraint are discussed in Chapter 3: (1) the interaction between temperature inactivation and dosage change during the cell cycle and (2) the enzyme activity – stability tradeoff. It was argued that functional differences of proteins and enzymes in the cell cycle might determine their thermodynamic properties. It may well be that it is very difficult to escape from this tight relationship between thermodynamics and function. This may also apply to dosage change during the cell cycle as a result of cell division and cell growth and the enzyme activity – stability tradeoff.

Genetics of plasticity

Chapter 4 showed that genetic variation in the biophysical parameters of the Sharpe – Schoolfield equation lead to both additive (mainly by variation in ρ and HA) and non-additive genetic (mainly by variation in inactivation parameters) variance in body size, in particular at extreme temperatures. The quantitative genetic basis of thermal reaction norms has been investigated with different several experimental designs and genetic variation for plasticity was generally found to be present. The variation among iso-females lines was used to investigate the genetic architecture of natural populations of *Drosophila melanogaster* (David *et al.* 1994, David, Legout & Moreteau 2006, Delpuech *et al.* 1995), a half-sib breeding design to investigate the genetics of egg size plasticity in a tropical butterfly (Steigenga *et al.* 2005), a split-sibship experiment to study the genetic and parental contribution to population differentiation in a temperate butterfly (Kingsolver *et al.* (2007) and selection lines to measure heritability of juvenile growth reaction norms in a collembolan insect (Driessen, Ellers & van Straalen 2006) and genotype by environment interaction for egg size and body size plasticity in a tropical butterfly (Fisher, Bauerfeind & Fiedler 2006). Genotype by environment variation was found in both butterfly species, implicating scope for evolutionary change, but not in the collembolan. The results from Chapter 4 suggest that a quantitative genetic analysis of thermal reaction norms might be carried out with estimated biophysical parameters used as quantitative characters. This may lead to a clearer separation of additive and non-additive effects and more insight in the proximate mechanisms.

A molecular genetic study of body size plasticity in the nematode *C. elegans* (Kammenga *et al.* 2007) showed that a subtle wild-type polymorphism modulates the temperature responsiveness of body size and suggested that size adaptation of ectotherms to temperature changes might be less complex than previously thought. Body size response to temperature in *C. elegans* is the result of cell size modulation as cell number is invariant in this species (see also van Voorhies 1996). These authors found a single nucleotide polymorphism in the calpain-like protease gene TRA-3. According to Kammenga *et al.* (2007), one wild-type strain obeys the TSR and another wild-type strain not. The naturally occurring mutation probably reduces the ability of TRA-3 to bind calcium. Their data indicate that calcium signalling in response to temperature changes may lead to the activation of TRA-3. This mechanism to control the TSR is supported by various reports on the elevation of the free cytosolic calcium concentration in response to lower temperatures. However, it is unclear which pathway links calcium activation of TRA-3 to larger cell sizes at lower temperatures in *C. elegans.* .Kammenga *et al.* 2007conclude that their results partly fits the Van der Have – de Jong model as this model presupposes that the TSR depends on a wide range of alleles differing in their sensitivity to temperatures. Their results show that a polymorphism in a single gene may attenuate the thermal reaction norm for body size in *C. elegans*. Interestingly, the wild type strain showing no TSR (in fact shows a slight, but not significant size reduction) was collected in Hawaii and the strain showing a significant TSR was collected in the UK. The Hawaii strain was significantly smaller than the UK strain, which conforms phenotypically to the Bergmann cline found in other ectotherms, although Bergmann clines are mainly caused by variation in cell number. This study was carried out at only two temperatures, and as mentioned before when discussing the paper of Kingsolver *et*

al. (2007), if the strains have different thermal limits and different thermal optima this may complicate the interpretation of their results. This does not affect, however, their conclusion that only one gene may determine the TSR in this species. Evidently, it would be very interesting to see if such simple polymorphisms may explain TSR in other ectotherms as well.

The aforementioned paper of Knies *et al.* (2006) studied the genetic basis of thermal reaction norms of a bacteriophage simply by sequencing the whole genome. Reaction norm shape was analyzed with a polynomial with three parameters, average growth rate, temperature of maximal growth and thermal range (Izem & Kingsolver 2005). They concluded that artificial adaptation to high temperatures was mainly the result of a horizontal shift, but there was a correlated response of a smaller thermal range (specialist – generalist shift) and a vertical shift. The latter would be expected from the influence of the Eyring equation, the smaller thermal range would be expected from the enzyme maximal activity – stability tradeoff. Again, the application of the Sharpe – Schoolfield equation might lead to more insight in the proximate mechanisms, and in particular if also sensitivity shifts were involved.

Any change in developmental timing is defined as heterochrony (Reilly, Wiley & Meinhardt 1997). Adaptation to lower temperatures generally leads to heterochrony as a result of the differential sensitivity of development and growth rate. Heterochrony is generally considered as a major source of evolutionary innovation and macroevolutionary change in animals (Gould 1977, McKinney & McNamara 1991) and is thought also to contribute the evolution of phenotypic plasticity (West-Eberhard 1989). It seems unlikely that a full symmetry exists in the temperature dependence of biological and biochemical processes in ectotherms (Somero 1991) and differential thermal sensitivity may rule rather than exception. Therefore, the quest is to search for functional and structural differences in temperature-dependence of the regulatory components of protein synthesis and metabolism (growth), cell cycle, differentiation and general development, physiology, and so on. The general or regulatory interaction of these processes will define the flexibility and limits of the temperature-dependence of the higher systemic levels.

Thermodynamic constraints?

Chapter 5 explored the effect of genetic variation in the biophysical parameters of the Sharpe- Schoolfield model. Table 6.1 summarizes the main effects of a change in enzyme characteristics and related genetic variation in the biophysical parameters on the phenotypic correlations (x-intercept or temperature threshold for development, slope – y-intercept correlation, Chapter 5), G X E interaction and genetic correlations between environments. Cases 1-3 refer to Figures 5.2A-C, respectively, and include a vertical and sensitivity shift (case 1), sensitivity shift (case 2) and vertical shift (case 3), case 4 refers to Figure 5.3 (a specialist – generalist shift) and case 5 to Figure 5.4.(a horizontal shift). A comparison with the polynomial analyses of Izem & Kingsolver (2005) and Knies *et al.* (2007) shows that the application of the Sharpe – Schoolfield equation can lead to a more detailed insight in the mechanisms underlying thermal adaptation.

Table 1. From enzymes to reaction norms to genetic correlation between environments. The case numbers are explained in the text, the parameters refer to the Sharpe – Schoolfield equation.

Case #	process	parameters	slope change	x-intercept change	slope-y-intercept correlation	G x E interaction present	Genetic Correlation sign change
1	increasing enzyme quantity	ρ	increase	no	no	yes	no
2	decreasing enzyme efficiency	H_A	increase	decrease	negative	yes	yes
3	decreasing efficiency & decreasing quantity	ρ, H_A	no	increase	no	no	no
4	increasing range of activity	T_L, T_H, H_L, H_H	decrease	decrease	negative	yes	yes
5	increasing optimum temperature	T_o, T_L, T_H	increase	increase	negative	yes	yes

The most important thermodynamic constraint is the enzyme activity – stability tradeoff (Hochachka & Somero 1984) that limits the viable development of ectotherms to a relative small range of ≈ 20 °C compared to the possible range of 100 °C. When ectotherms adapt to lower temperatures (horizontal shift) it is likely that a correlated response occurs of a wider thermal range (specialist – generalist shift), a smaller slope (sensitivity shift) and lower activity (vertical shift). This correlated response is mainly determined by the Eyring equation, which can therefore explain much of the variation in thermal characters among species. But is this really a constraint? Within thermal environments all ectotherms share this constraint, which implies that it does not severely limit evolution within thermal environments. On the contrary, adaptation to different parts of the Eyring equation by relatively small changes in thermal limits will lead to relatively large changes in phenotypes which in combination with thermal habitat selection may promote co-existence of different genotypes and, by implication, speciation. In this view the Eyring equation does not limit selection and evolution, but instead may be one of the drivers of evolution and consequently biodiversity.

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SUMMARY

Introduction
Temperature strongly influences the phenotype of ectotherms and many species differ in their sensitivity to temperature. The viable temperature range might be narrow or wide, and body size often changes with development temperature. Ectotherm development rate depends strongly on temperature, while growth rate does so to a lesser extent. Development rate and growth rate show a more or less triangular shape with temperature, rising slowly and almost linearly with temperature to a maximum rate at a fairly high temperature, and decreasing steeply after at higher temperatures. The size at maturity in many ectotherms decreases with increasing temperature over a large part of the viable temperature range. This thesis investigates how thermodynamics proximately determine biological rates and to what extent it ultimately constrains thermal adaptation of developing ectotherms. The biophysical Sharpe – Schoolfield model, which connects enzyme kinetics and biological rates, is successfully applied to three important issues in evolutionary ecology: the temperature dependence of body size in ectotherms, temperature tolerance limits in developing ectotherms and patterns of thermal adaptation within and among species.

Adult size in ectotherms
Chapter 2 proposes a proximate, biophysical model to describe temperature-modulated variation in growth rate and differentiation rate in ectotherms. The model assumes 1) that growth rate and differentiation rate can be described as controlled by one rate-limiting enzyme; in addition, the model assumes 2) that the temperature coefficients of growth and differentiation are different. The model is used to predict temperature-dependent size variation at maturation of ectotherms as a result of the interaction of growth and differentiation. It is shown that the difference between the activation energy constants of growth and differentiation determines the slope of the size - temperature reaction norm within the range of normal development. The structural and heritable variation in enzymes determines reaction norm shape without inferring regulatory genes. All thermodynamic parameters of the Sharpe-Schoolfield equation can be estimated empirically with non-linear regression techniques. The biophysical model provides a proximate framework for genotypic models of reaction norm evolution; genetic variation in either growth or differentiation would lead to genotype by environment interaction. This proximate model of temperature sensitivity and temperature tolerance clarifies how temperature dependence of body size would evolve.

Thermal tolerance limits
Thermal limits of viable ectotherm development are threshold-like and near symmetrical around the temperature of optimal performance and usually well within the thermal tolerance range of adult physiological traits. A proximate model is proposed in Chapter 3 to show that the interaction between reversible temperature inactivation of cell cycle proteins and their regulation can explain (1) the symmetry and (2) threshold character of thermal limits of viable embryonal and larval development in ectotherms. It is suggested that temperature inactivation of regulatory proteins mimics the decrease in concentration

resulting from gene dosage change and transcriptional regulation during the cell cycle. If certain regulatory proteins have equal probability to be active or inactive at a certain temperature, cell division and, consequently, development becomes blocked. Model predictions were tested by comparing thermal tolerance limits as observed in viability experiments with 14 developing insect species with the estimated temperatures at which a hypothetical rate-determining developmental enzyme has an equal probability to be active or inactive. These 'expected' thermal limits were derived from the Sharpe-Schoolfield equation, which describes temperature-differentiation rate reaction norms. In 21 out of 23 comparisons 'expected' thermal limits agree closely with the observed thermal tolerance limits. The implications of the model for thermal tolerance, thermal adaptation, epidemiology and life-history strategies are discussed.

From biophysics to adaptation

Many models have been proposed to describe the temperature dependence of development rate, from the linear degree-day summation to a non-linear model based upon biophysics. Chapter 4 reviews the degree-day model and the biophysical Sharpe-Schoolfield model. The latter model is preferred as it has a clear biophysical base, and provides an accurate description of the temperature dependence of biological rates. It can be used to describe phenotypic plasticity in development rate, growth rate and ectotherm size. Any change in parameters in the model immediately explains why genetic variation for phenotypic plasticity can be found. The optimal temperature for organismal functioning is part of the model. This optimal temperature proves not to be identical to the temperature of highest development rate or highest growth rate. Some of the biophysical parameters in the model can predict the boundaries of the viable temperature range. It is argued that by applying the Sharpe-Schoolfield model, biophysics can be used to explain size differences over temperatures and geographical clines in adult body size. Selection on development rate or growth rate would translate into selection on the parameters of the model. So would selection for enzyme efficiency or enzyme stability. The Sharpe-Schoolfield model can therefore be used to link adaptation at the physiological level to phenotypic plasticity in body size. We can see why phenotypic plasticity is adaptive, or not, what traits are the prime movers of adaptation and what traits might be easily observed but not be adaptive themselves.

Thermal adaptation in ectotherms

Chapter 5 investigates how patterns in thermal adaptation within and among species can be modelled and predicted from the Eyring equation and Sharpe – Schoolfield model that is based on the kinetics of reaction rates and enzyme properties. The tangent of the general temperature dependence among species described by the Eyring Equation can approximate the slope of linear, within-species thermal reaction norms. The linearity itself is caused by reversible temperature inactivation. The thermal time model complies with the linear part of the developmental rate – temperature reaction norm described by the Sharpe - Schoolfield model.

It is shown that small changes in the parameter variation in the biophysical Sharpe - Schoolfield model can generate all three major patterns of thermal adaptation and in addition a fourth pattern, namely, a sensitivity shift or variation in the slope of the thermal reaction norm. A shift in sensitivity can result directly from variation in reference

rate or activation energy, and indirectly from a shift in thermal range or optimal temperature. Therefore, a phenotypic shift in sensitivity is not informative about the underlying change in thermal parameters included in the Sharpe – Schoolfield model.

If only the development rate at the reference temperature is varied, and all thermal parameters are kept constant, the slope of the linear part will vary with the reference rate but the threshold temperature will remain constant. This situation applies to developmental rate isomorphy, the observation that in many insect species the temperature sensitivity varies with developmental stages but the threshold temperature h remains constant. It is proposed that the Sharpe – Schoolfield model provides a mechanistic explanation for developmental rate isomorphy.

Finally, the variation in thermal reaction norms in a large number of anurans based on published datasets is analysed. In about 50 species most thermal parameters, such as reference rate, optimal temperature, thermal range and thermal tolerance limits have been experimentally measured. All four patterns of thermal adaptation occur in this group of related anurans, but that most variation in ρ and slope can be explained by the combination of a horizontal shift (hotter – colder) and a response following the Eyring equation. The implications of the results are discussed in relation to the assumptions of the proposed Universal Temperature Dependence, the correlation between egg size and development rate and the evolutionary optimisation of egg size in relation to thermal environment.

Conclusion

When ectotherms adapt to lower temperatures (horizontal shift) a correlated response occurs of a wider thermal range (specialist – generalist shift), a smaller slope (sensitivity shift) and lower activity (vertical shift). This correlated response is mainly determined by the Eyring equation. The enzyme activity – stability tradeoff is the most important thermodynamic constraint and limits the viable development of most ectotherms to a relative small thermal tolerance range of approximately 20 °C. It is argued that this correlated response does not limit evolution within thermal environments, but instead may be one of the drivers of evolution and consequently biodiversity. The overall conclusion is that the biophysical Sharpe – Schoolfield equation is an excellent model to study thermal adaptation in ectotherms.

SAMENVATTING

Inleiding
Temperatuur beïnvloedt heel sterk de uiterlijke verschijningsvorm (het fenotype) van koudbloedige organismen (ectothermen) en veel soorten verschillen in hun gevoeligheid voor temperatuur. De temperatuurrange waarbinnen organismen levensvatbaar kunnen ontwikkelen kan smal zijn of breed en lichaamsgrootte verandert vaak met de ontwikkelingstemperatuur. De ontwikkelingssnelheid van ectothermen hangt ook sterk af van de temperatuur, terwijl groeisnelheid daar in mindere mate afhankelijk van is. Zowel ontwikkelingssnelheid als groeisnelheid neemt geleidelijk vrijwel lineair toe tot een maximum, maar neemt daarboven sterk af. De volwassen lichaamsgrootte neemt in veel ectothermen af met toenemende temperatuur over het grootste deel van levensvatbare temperatuurrange. Dit proefschrift onderzoekt hoe proximate, thermodynamische processen biologische snelheden bepalen en in hoeverre deze processen beperkend kunnen zijn voor de ultimate temperatuuraanpassing van ontwikkelende ecothermen. Het biofysische Sharpe – Schoolfield model, welke een verbinding legt tussen enzym kinetica en biologische snelheden, is met succes toegepast op drie belangrijke thema's in de evolutionaire ecologie van ectothermen: de temperatuurafhankelijkheid van lichaamsgrootte, de temperatuur tolerantiegrenzen en de patronen van temperatuuraanpassing binnen en tussen soorten.

Volwassen lichaamsgrootte bij ectothermen
Hoofdstuk 2 stelt een proximaat, biofysisch model voor om temperatuur gemoduleerde variatie in groeisnelheid en differentiatiesnelheid in ectothermen te beschrijven. Het model neemt aan dat (1) groeisnelheid en differentiatiesnelheid kunnen worden beschreven als ware zij controleert door een snelheidsbeperkend enzym, en (2) de temperatuurcoëfficiënten van groeisnelheid en differentiatiesnelheid zijn verschillend. Het model wordt gebruikt om de temperatuurafhankelijke variatie in volwassen lichaamsgrootte te voorspellen die voortkomt uit de interactie tussen groei en differentiatie. Aangetoond wordt dat het verschil tussen de activatie-energieën van groeisnelheid en differentiatiesnelheid de hellingshoek bepaalt van de temperatuur – lichaamsgrootte reactienorm binnen de temperatuurrange van normale ontwikkeling. De structurele en erfelijke variatie in enzymen bepalen de vorm van de reactienorm zonder de betrokkenheid van regulatiegenen. Alle thermodynamische parameters van de Sharpe – Schoolfield vergelijking kunnen worden geschat met niet-lineaire regressie technieken. Het biofysische model voorziet in een proximaat kader voor genotypische modellen van reactienormevolutie. Genetische variatie in groei of differentiatie leidt tot genotype bij omgevingsinteractie. Dit proximate model voor temperatuurgevoeligheid en temperatuurtolerantie verklaart hoe temperatuurafhankelijkheid van volwassen lichaamsgrootte bij ectothermen kan evolueren.

Grenzen van temperatuurtolerantie
De grenzen van temperatuurtolerantie van de levensvatbare ontwikkeling van ectothermen hebben het karakter van een drempelwaarde en liggen meestal symmetrisch rond de optimale temperatuur en ruim binnen de tolerantiegrenzen van fysiologische kenmerken van het volwassen organisme. In hoofdstuk 3 wordt een proximaat model

voorgesteld dat door de interactie tussen de omkeerbare temperatuurinactivatie van celcyclus eiwitten en hun regulatie (1) de symmetrie en (2) het drempelwaardenkarakter van de tolerantiegrenzen van ontwikkelende ectothermen kan verklaren. De reversibele inactivatie van regulatie-eiwitten heeft hetzelfde effect als de afname in concentratie als gevolg van gendosis verandering en transcriptieregulatie gedurende de cel cyclus. Als bepaalde regulatie-eiwitten nog maar half actief zijn bij een bepaalde temperatuur, dan wordt stopt de celdeling en als gevolg daarvan de ontwikkeling van het organisme. De voorspellingen van het model werden getoetst aan de empirische bepaalde temperatuurtolerantiegrenzen van veertien soorten insecten. De verwachte tolerantiegrenzen werden berekend met de Sharpe – Schoolfield vergelijking. In 21 van de 23 vergelijkingen bleken de verwachte en gevonden waarden sterk overeen te komen. De implicaties van het model voor temperatuuraanpassing, epidemiologie en levensgeschiedenisstrategieën worden besproken.

Van biofysica naar adaptatie

Veel modellen zijn voorgesteld om de temperatuurafhankelijkheid van ontwikkelingsnelheid te beschrijven, variërend van het lineaire, temperatuur-dagsom model tot lineaire modellen gebaseerd op biofysica. Hoofdstuk 4 geeft een overzicht van het temperatuur-dagsom model en het Sharpe – Schoolfield model. Het laatste model heeft de voorkeur omdat het een duidelijke biofysische basis heeft en een zeer accurate beschrijving geeft van de temperatuurafhankelijkheid van biologische processen. Het kan gebruikt worden om de fenotypische plasticiteit te beschrijven van ontwikkelingsnelheid, groeisnelheid en volwassen lichaamsgrootte in ectothermen. Elke verandering in de model parameters kan direct verklaren waarom genetische variatie in fenotypische plasticiteit wordt gevonden. De optimale temperatuur voor het functioneren van organismen is een onderdeel van het model. Deze optimale temperatuur blijkt niet gelijk aan de temperatuur waarbij ontwikkelingsnelheid of groeisnelheid het hoogst is. Met het toepassen van het Sharpe – Schoolfield model kan biofysica gebruikt worden om de temperatuurafhankelijkheid van en clinale geografische variatie in lichaamsgrootte te verklaren. Selectie op groeisnelheid en ontwikkelingsnelheid kan vertaald worden in selectie op de parameters van het model, net zoals dat het geval is voor de selectie voor enzymefficiëntie en enzymstabiliteit. Het Sharpe – Schoolfield model kan daarom gebruikt worden om adaptatie op het fysiologische niveau te koppelen aan de fenotypische plasticiteit van lichaamsgrootte. Het wordt daardoor duidelijk wanneer fenotypische plasticiteit adaptief is of niet, welke kenmerken verantwoordelijk zijn voor het ontstaan van adaptatie en welke kenmerken dat ten onrechte lijken te zijn.

Temperatuuradaptatie van ectothermen

Hoofdstuk 5 onderzoekt hoe patronen in temperatuuraanpassing binnen en tussen soorten voorspeld kunnen worden op basis van de Eyring vergelijking en het Sharpe – Schoolfield model dat gebaseerd is op de kinetica van reactiesnelheden en enzymeigenschappen. De raaklijn van de exponentiële Eyring vergelijking, gebaseerd op een vergelijking tussen soorten, bij de optimale temperatuur is een goede benadering van de hellingshoek van de lineaire, soortafhankelijke reactienorm. Het lineaire deel zelf wordt veroorzaakt door reversibele temperatuurinactivatie van enzymen. Het temperatuur-dagsom model is goed vergelijkbaar met het lineaire deel van de

temperatuur – ontwikkelingsnelheid reactienorm beschreven door het Sharpe – Schoolfield model. In het hoofdstuk wordt aangetoond dat kleine veranderingen in de parametervariatie van het model de drie belangrijkste patronen in temperatuuraanpassing kan genereren en aanvullend een vierde patroon, namelijk een *shift* in de temperatuurgevoeligheid ofwel variatie in de hellinghoek van de reactienorm. Een verandering in de temperatuurgevoeligheid kan het directe resultaat zijn van variatie in de referentiesnelheid bij de optimale temperatuur of activatie-energie en indirect het gevolg van een verandering in de temperatuurrange of optimale temperatuur. Een fenotypische verandering in de temperatuurgevoeligheid geeft daarom geen direct inzicht in het onderliggende, biofysische proces.

Indien alleen de referentiesnelheid bij de optimale temperatuur verandert, en alle andere parameters constant blijven, dan varieert de hellingshoek van het lineaire deel van de reactienorm, maar de drempelwaarde-temperatuur voor ontwikkeling blijft constant. Dit verschijnsel is bij insecten bekend als ontwikkelingsnelheid - isomorfie. Dit heeft betrekking op de waarneming dat in veel insectensoorten de temperatuurgevoeligheid van verschillende ontwikkelingstadia varieert, maar dat de drempelwaarde - temperatuur voor ontwikkeling constant blijft. Het Sharpe – Schoolfield model kan voor dit verschijnsel een mechanistische verklaring geven.

Als laatste wordt de variatie in temperatuur reactienormen geanalyseerd van een groot aantal soorten padden en kikkers op basis van gepubliceerde datasets. In ongeveer vijftig soorten zijn een experimenteel groot aantal parameters bepaald die betrekking hebben op temperatuuraanpassing, zoals ontwikkelingsnelheid, optimale temperatuur en temperatuurtolerantiegrenzen. Alle vier patronen in temperatuuraanpassing komen voor in deze groep van verwante padden en kikkers, maar dat de meeste variatie in de ontwikkelingsnelheid bij de optimale temperatuur en de hellingshoek verklaard kan worden door een combinatie van een horizontale *shift* (warmer – kouder) en een gecorreleerde response in hellingshoek en temperatuurtolerantierange bepaald door de Eyring vergelijking. De implicaties van deze resultaten worden bediscussieerd in relatie tot de aannames van het recent voorgestelde model voor de Universele Temperatuurafhankelijkheid van groeisnelheid en ontwikkelingsnelheid.

Conclusie

Als ectothermen zich aanpassen aan lagere temperaturen (horizontale *shift*) dan treedt er een gecorreleerde respons op in de temperatuurtolerantierange (specialist – generalist *shift*), een lagere hellingshoek van de reactienorm (*shift* in gevoeligheid) en een lagere activiteit (verticale *shift*). Deze gecorreleerde respons wordt vooral bepaald door de Eyring vergelijking. De *tradeoff* tussen enzymactiviteit en -stabiliteit is de belangrijkste thermodynamische *constraint* en beperkt de levensvatbare ontwikkeling van de meeste ectothermen tot een relatief smalle temperatuur tolerantierange van ongeveer 20 ˚C. De gecorreleerde respons is niet zozeer beperkend voor de evolutie in een veranderende thermische omgeving, maar kan juist een aanjager zijn van evolutionaire processen. De algemene conclusie is dat het biofysische Sharpe – Schoolfield model een excellent model is om temperatuuraanpassing bij ectotherme organismen te bestuderen.

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CURRICULUM VITAE

Thomas Marinus van der Have was born on 8 September 1956 in Amsterdam, The Netherlands. He obtained his gymnasium β diploma in Amstelveen and graduated in Biology (*cum laude*) at the Free University, Amsterdam, January 1984 with majors on the evolutionary genetics of social insects and ecology of waders. In 1984 he obtained a British Council FCO Special Award Scholarship and worked as a post-graduate research fellow at Furzebrook Research Station, ITE, UK, and University of Aarhus, Denmark, to continue to work on the evolutionary ecology of coastal dune ants. Since 1985 he works together with Prof. dr. J.J. (Koos) Boomsma, first at the Department of Population Biology and Evolution, University Utrecht on sex ratio evolution in ants and later at Aarhus University and Copenhagen University on paternity skew in ants. From 1987 to 1993 he continued to work as a research fellow at Utrecht University on the evolutionary genetics of reaction norms with Dr. Gerdien de Jong. In recent years their research interest focused on theoretical aspects of thermal adaptation in ectotherms.

From 1994 to 2003 he worked as a conservation biologist at Vogelbescherming Nederland on species and habitats, including projects on spoonbills, white storks and coastal birds. As a science co-ordinator he participated in the Steering Committee for the research program evaluating the coastal shellfisheries policy. Since 2004 he works in in the Centre for Ecosystem Studies, Wageningen University, first as centre co-ordinator and subsequently as project leader in the chairgroup Resource Ecology Group of Prof. dr. Herbert Prins. Together with Prof. dr. Steven de Bie, chair Sustainable Use of Living Resources, he currently develops the post-MBA track “Creating Value, New frontiers in Corporate Responsibility and Leadership” in co-operation with TiasNimbas Business School, Tilburg University. From 2005 onwards he is also working as a science co-ordinator at the Faunafonds, Dordrecht, to set up a research program aimed at preventing and managing agricultural damage caused by protected species.

Besides his research interest in evolutionary ecology of ants and thermal adaptation of ectotherms, he is since long interested in the migration ecology of shorebirds and rare birds. He participated in and organised twelve international expeditions to wetlands in Africa, Mediterranean and Middle East, including studies of Broad-billed Sandpiper and the near-extinct Slender-billed Curlew, and discovered two species new for The Netherlands (Yellow-rumped Warbler, Pied Wheatear). He is chairman of the Foundation Working Group International Waterbird and Wetlands Research. He is married to Nicoline ter Hoeve and father of two sons, Auke and Sybe.

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PE&RC PHD EDUCATION CERTIFICATE

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of Literature (5.6 ECTS)
- Temperature-size rule: one model to rule them all? (2007)

Writing of Project Proposal (7 ECTS)
- Modelling temperature dependence of life-history characters in developing ectotherms (2006)

Post-Graduate Courses (1.4 ECTS)
- EURING Technical Workshop Statistical Analysis of Marked birds; EURING, U.E.A., Norwich, UK (1997)

Competence Strengthening / Skills Courses (2.6 ECTS)
- Beter presenteren; BirdLife (2001)
- Groene wetgeving; Stichting Instituut Voor Agrarisch Recht (2001)
- Omgaan met de opdrachtgever; ESG (2004)

Discussion Groups / Local Seminars and Other Meetings (7.2 ECTS)
- Annual Meeting International Wader Study Group; 12 meetings of two days (1987-2004)

PE&RC Annual Meetings, Seminars and the PE&RC Weekend (0.9 ECTS)
- Seminar Ecosystems Response to Climate Change (2004)
- Kick-off meeting Animal Network Research Group; REG (2007)

International Symposia, Workshops and Conferences (21 ECTS)
- 1st Congress European Society for Evolutionary Biology; Basel, Switzerland (1987)
- 10th International Congress IUSSI.; München, Germany (1987)
- International Ornithological Congress; Durban, South Africa, invited lecture (1998)
- 2nd Congress European Society for Evolutionary Biology, Rome, Italy (1989)
- 20th Conference European Society for Comparative Physiology and Biochemistry; Aarhus, Denmark, invited lecture (1999)
- 10th International Wadden Sea Scientific Symposium; Groningen, the Netherlands (2000)
- 3rd Conference European Ornithologist Union; Groningen, the Netherlands (2001)

Colophon

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