Adult Size in Ectotherms: Temperature Effects on Growth and Differentiation

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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 of ectotherms at maturation 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. 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 nonlinear 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.

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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 norms of growth rate, development rate and size at maturity as a function of temperature, focusing on 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).

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Recent empirical work (e.g. Reznick, 1990, 1993) 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, 1995), 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 & 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 the 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. Here, we are not concerned with diversification, but with the increase in cell number during development: this is the aspect of differentiation referred to. 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 differentiation. 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, 1993a,b; 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, 1993a,b). 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 molt. 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 fully
grown organisms has been well studied in *Drosophila* and found to be influenced by environmental conditions during development at a 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) their temperature coefficients can be 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:

\[ m = m_0 + Gt \]  

(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:

\[ m = m_0 + \frac{G}{D}. \]  

(2)

**SHARPE–SCHOOLFIELD EQUATION**

Both growth rate and differentiation 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:

\[ r(T) = \frac{\rho T P_T}{298.2} \exp \left( \frac{\Delta H^*_f}{R} \left( \frac{1}{298.2} - \frac{1}{T} \right) \right) \]  

(3)

where \( r(T) \) is the development rate (days\(^{-1} \)) at temperature \( T(°K) \), \( P_T \) is the probability that the rate controlling enzyme (of development) 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: \( \rho \) 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), \( \Delta H^*_f \) is the enthalpy of activation (cal mol\(^{-1} \)) (Hochachka & Somero, 1984) of the rate controlling enzyme. The probability \( P_T \) that the rate controlling enzyme is in active state is defined by:

\[ \frac{1}{P_T} = 1 + \exp \left( \frac{\Delta H^*_f}{R} \left( \frac{1}{T_{1/2_a}} - \frac{1}{T} \right) \right) + \exp \left( \frac{\Delta H^*_f}{R} \left( \frac{1}{T_{1/2_i}} - \frac{1}{T} \right) \right) \]  

(4)

where \( T_{1/2_a} \) and \( T_{1/2_i} \) are the temperatures (°K) at which the enzyme is half active and half inactive by high or low temperatures, respectively, and \( \Delta H^*_f \) and \( \Delta H^*_i \) are the changes in enthalpy (cal mol\(^{-1} \)) associated with respectively high or low temperature inactivation of the enzyme.

The Sharpe-Schoolfield equation has considerable advantages over other expression of biological rate functions (reviewed in Wagner et al. (1984). It describes accurately the temperature dependence of a developmental process over the whole range of biological activity, including the quasi-linear region at intermediate temperatures and the nonlinear regions at high and low temperatures (Fig. 1). Reversible inactivation of enzymes approximately linearizes the exponential rate function expected from the Eyring
Genetic variation in the enthalpy of activation, \( \Delta H^a \), for 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, \( mt^{-1} \)) and differentiation rate \( D \) (developmental stages per unit time, \( r^{-1} \)) are expressed as functions of temperature according to eqn (3) and substituted in eqn (2), mass at metamorphosis as a function of temperature \( T \) will be:

\[
m(T) = m_b + \frac{\rho_D}{\rho_G} \exp\left(\frac{\Delta H^a - \Delta H^c}{R} \left(\frac{1}{298.2} - \frac{1}{T}\right)\right)
\]  

(5)

where the subscripts refer to growth rate \( g \) and differentiation rate \( d \). \( \rho_G \) is growth rate (mass·time\(^{-1}\)) and \( \rho_d \) 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.

Smaller size at metamorphosis or maturation in ectotherms grown at higher temperatures but under surplus food conditions is a widely observed phenomenon (Bélehradék, 1935; von Bertalanffy, 1960; Ray, 1960; Precht et al., 1973; Atkinson, 1994a,b; 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 eqn (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 \( T_{1/2_{l,d}} \) and \( T_{1/2_{l,g}} \). Then, size at metamorphosis will be a decreasing function of temperature if the following inequalities are satisfied:

\[
\Delta H^a_{l,d} - \Delta H^c_{l,d} > (1 - P_L)\Delta H^a_{l,g}
\]

(6a)

\[
- (1 - P_L)\Delta H^c_{l,g} \quad \text{(for } T > 298.2°K) \]

\[
\Delta H^a_{l,d} - \Delta H^c_{l,d} > (1 - P_L)\Delta H^a_{l,g}
\]

(6b)

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- (1 - P_L)\Delta H^c_{l,g} \quad \text{(for } T < 298.2°K) \]

where \( P_L \) is the inactivation parameter at the reference temperature. If both growth rate \( G \) and differentiation rate \( D \) are expressed as functions of temperature according to eqn (3), mass at metamorphosis as a function of temperature \( T \) will be:

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\]

(6b)

\[
- (1 - P_L)\Delta H^c_{l,g} \quad \text{(for } T < 298.2°K) \]

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m(T) = m_b + \frac{\rho_D}{\rho_G} \exp\left(\frac{\Delta H^a - \Delta H^c}{R} \left(\frac{1}{298.2} - \frac{1}{T}\right)\right)
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(5)

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\]

(6b)

\[
- (1 - P_L)\Delta H^c_{l,g} \quad \text{(for } T < 298.2°K) \]
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 $P_1$ 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) and (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 ectothermic 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 is that growth rate and differentiation rate have different temperature coefficients. Hochachka & 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 eqn (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 $\Delta H_{T+d}^{\text{eff}}$ of growth should be lower than the temperature coefficient $\Delta H_{T}^{\text{eff}}$ 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 1500000 and 3000000, respectively) in comparison with the much smaller DNA polymerases (molecular weights between 110000 and 1800000). This implies that the large ribosomal subunits diffuse slower into the cytoplasm, before assembly 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 ($Q_w$ close to one, Hochachka, 1991). DNA replication will depend upon the enzymatic speed of the polymerases, with a $Q_w$ nearer to two. 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, 1978). Egg development (mainly differentiation) was found to be more sensitive to temperature than larval growth (mainly protein synthesis).

In the model the simplest possible type of growth is chosen as the second 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 $m = m_0 e^{\gamma t}$, where $\gamma$ is the instantaneous growth rate with temperature dependence following eqn (3), leads to essentially the same conditions [eqns 6(a,b)] for the slope of the size–temperature reaction norm to be negative. It should be noted in this context that nonlinear 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, nonlinear models are indistinguishable in performance from exponential or even linear models to describe growth (Reiss, 1989).

The third assumption, that one reaction step is rate limiting, one to growth and one to differentiation, is already present in the Sharpe–Schoolfield eqn (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.

A fourth assumption of the biophysical model is that the focal enzyme in eqn (3) occurs reversibly in active or inactive state. The optimal temperature at which little or no inactivation occurs, is arbitrarily chosen as 25°C (298.2°K, Schoolfield et al., 1981), although any temperature between 20° and 30° would be appropriate for most organisms. A particular realistic property of eqn (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 & Kingsolver, 1989; Huey et al., 1991; e.g. ovariole number and egg production in Drosophila, David et al. (1983)].

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 Sharpe–Schoolfield equation (3) are substituted in eqn (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., 1981; David & Clavel, 1967; respectively). To illustrate the usefulness of eqns (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.

The thermodynamic constants of differentiation and growth rates were estimated with nonlinear 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). Figure 4 shows the temperature dependence of weight (size) at

![Graph showing 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 refer to the observed thermal limits of larval development. Data from Cohet et al. (1981) and David & Clavel, (1967).](image)

**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>$\rho_{\text{days}^{-1}}$</th>
<th>$\Delta H_f^{\text{cal mol}^{-1}}$</th>
<th>$T_{1/2}^{\text{K}}$</th>
<th>$\Delta H_f^{\text{cal mol}^{-1}}$</th>
<th>$T_{1/2}^{\text{K}}$</th>
<th>$\Delta H_f^{\text{cal mol}^{-1}}$</th>
</tr>
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<tr>
<td>Differentiation rate</td>
<td>0.136</td>
<td>15789</td>
<td>285.3</td>
<td>42232</td>
<td>305.3</td>
<td>52730</td>
</tr>
<tr>
<td>Growth rate</td>
<td>0.160</td>
<td>9465</td>
<td>287.3</td>
<td>59455</td>
<td>305.4</td>
<td>138164</td>
</tr>
</tbody>
</table>
eclosion if all estimated thermodynamic parameters are substituted into eqn (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 32°C, respectively).

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 (1993a) 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. 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 et al., 1991), 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 nonlinear regression methods on the basis of eqn (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). This shows the Sharpe–Schoolfield equation (3) to be a securely founded model to describe developmental rates.

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 [eqn 6(a) and (b)]; these conditions define the central problem of this paper. Variation in the change of enthalpy by high and low temperature inactivation (respectively $\Delta H^a$ and $\Delta H^c$) mainly influences the shape of the nonlinear 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 $P_s = P_d = 1$; 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 $\Delta H^c_d - \Delta H^c_e$ 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 of metamorphosis with temperature, within the range of normal development. This property can be illustrated in the six-parameter model if either $\Delta H^c_d$ or $\Delta H^c_e$ 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 by environment interaction in size at metamorphosis.

What are the implications for quantitative genetic models of 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 in a simple mathematical model to explain final size in a study of the cladoceran Simocephalus vetulus or asymptotic body size in a study of the cladoceran Daphnia galeata. Third, it suggests that the temperature coefficient of absorption and metabolism, as the first depends more on physical processes like permeation and diffusion, the second being more of a chemical nature (Von Bertalanffy, 1960; Precht et al., 1973; Ratte, 1984; Ray, 1960; Atkinson, 1994, 1995 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 (here supposed to depend mainly on DNA replication during the cell division cycle) is higher than of growth (here supposed to depend mainly 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, 1995), few proximate explanations have been put forward.

Von Bertalanffy (1960) 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, 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

**Fig. 5.** Size at metamorphosis as a function of temperature [eqn (5)] with varying values for $H^*_{A,d} - \Delta H^*_{A,g}$: a small difference produces a shallow slope and a large difference leads to a steep slope in the size-temperature reaction norm. **Key:** $\Delta T_f$ — 9500; 8500; 7500; 6500; 5500; 4500; 3500; 2500; 1500; 500; 100.
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 TIMESCALE

Van Straalen (1983) suggested an operational definition of a physiological timescale (sensu Taylor, 1981). He noted that, if temperatures varies, a common physiological timescale 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 nonlinearly at extreme temperatures according to the Sharpe–Schoolfield equation and may have intrinsically different temperature coefficients. This severely limits the application of physiological timescales to temperature variation in life-history characters, as is implied by the arguments of van Straalen (1983). One could even conclude that physiological timescales 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 timescale.

Any change in size at metamorphosis or maturation as a result of a change in developmental timing is by definition heterochrony, irrespective of the timescale 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, 1993a,b; de Jong, 1990, 1995) and an increasing number of studies reported genetic variation in reaction norms (e.g. Weis & Gorman, 1990). 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, 1995) comprehensively reviewed temperature modulated variation in final size in organisms ranging from plants and protists to ectothermic animals, and concluded that no single overriding ecological or adaptive explanation has been proposed that could account for the almost 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; the biophysical mode presents the scope for adaptive variation to evolve in its parameters. The conditions [eqn (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 of 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 a general adaptive explanation for size reduction at higher temperatures. 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 shorter development time at higher temperatures, their paper clearly shows how difficult it is to find one 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. 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, 1997). From this assumption the same effect of temperature on oocyte production as on size at metamorphosis could be predicted, that is, smaller eggs will be produced at higher environmental temperature. 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 (1997); 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). The interaction between growth and differentiation defines another constraint: an intrinsic difference in the temperature coefficients of differentiation (cell number, cell division, DNA replication) and growth (biomass, cell size, protein synthesis) determines the reaction of ectotherm body size to environmental temperature. That reaction of body size 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 adaptive growth and adaptive differentiation can easily be put to adaptive use, leading to overall adaptive body size.

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