Temperature dependence of development rate and adult size in *Drosophila* species: biophysical parameters

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Abstract

Adult size in *Drosophila* results from the ratio of the rate of biomass increase and the rate of differentiation, both rates being temperature sensitive. Data on rates and size are collected in two tropical and two temperate *Drosophila* species; differentiation rate is higher in the two tropical species, growth rate differs between the large and small species of similar climatic origin. A biophysical model is used to evaluate the temperature dependence of adult size in *Drosophila*. The model is based upon the Sharpe–Schoolfield equation connecting enzyme kinetics and biological rates. Temperature sensitivities of growth rate, development time, and wing and thorax size are characterized by biophysical parameters. The biophysical parameter indicating trait specific temperature sensitivity is lower in tropical species than in temperate species, both for growth rate and for differentiation rate. In the larger species of a climate pair, thorax size and wing size prove to differ in pattern of temperature dependence; in the smaller species of a geographical pair, thorax size and wing size have identical patterns of temperature dependence.

Introduction

Body size is a key trait in life-history evolution, mediating increase and decrease of fitness by summarizing different fitness components. Body size of a large majority of ectotherm organisms is larger at lower temperatures, although development rate is slower (Atkinson, 1994). However, studies on different *Drosophila* species have shown that over a complete thermal range, size is some function of temperature with an intermediate maximum (David *et al*., 1994; Morin *et al*., 1996, 1997; Moreteau *et al*., 1997; Karan *et al*., 1998). Rather than explaining smaller flies at high temperature and bigger ones at low temperatures, we have to find a theoretical interpretation of the pattern of changes in size with temperature. In order to do this, we will examine a biophysical explanation and apply a biophysical model to data from four species of *Drosophila*.

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Development consists of two different components that can evolve independently: growth and differentiation. Growth equals increase in biomass, and growth rate has the dimension of biomass per unit time. The reciprocal of development time is the differentiation rate. Growth rate and differentiation rate together determine the size of the organism, as has been initially proposed for size at metamorphosis in Amphibia (Berven *et al*., 1979; Smith-Gill & Berven, 1979). These authors presented data indicating that growth rate and differentiation rate are controlled by different and functionally separate mechanisms. Insect size is widely regarded as resulting from the ratio of growth rate and differentiation rate. Bradshaw & Johnson (1995) and Gotthard & Nylin (1995) explain life-history implications of insect size under different food conditions as a consequence of changes in growth rate and differentiation rate. As almost all biological rates, both rates are temperature sensitive. Temperature sensitivity of insect size is explained by Ernsting & Isaaks (1997) and Gotthard & Nylin (1995) as a consequence of differences in temperature sensitivity of growth rate and differentiation rate. Gilbert & Raworth (1996) used the difference in temperature sensitivity of growth rate and differentiation rate as an explanatory

principle to adaptive life histories in insects. Van der Have & de Jong (1996) proposed a biophysical model of growth rate and differentiation rate in order to quantify the temperature dependence of adult size, the ratio between growth rate and differentiation rate; their biophysical model for temperature dependence of body size is based upon the Sharpe–Schoolfield equation (Sharpe & DeMichele, 1977; Schoolfield *et al*., 1981) describing temperature sensitivity of biological rates. Quantification of temperature sensitivities of growth rate and differentiation rate, and the resulting temperature sensitivity of adult size, will contribute to our insight in insect life history adaptations.

To collect an initial set of data to start thinking on this problem, four species of *Drosophila* with different thermal ranges were chosen to start comparisons over species. The two tropical species are *D. ananassae* and *D. willistoni*, the two temperate species *D. funebris* and *D. subobscura*. The species differ moreover in size: *D. ananassae* is the larger of the tropical species and *D. funebris* of the temperate species.

We investigated the suitability of the biophysical model of van der Have & de Jong (1996) for the study of temperature sensitivity in *Drosophila*. The aim is to compare temperature coefficients between species from different climatic zones that consequently differ in temperature range of development, in order to gain insight into the biological structure of temperature sensitivity of growth rate, development time and body size in *Drosophila* species. Conceivably, steeper increase in rates with temperature could be expected in temperate species, to make full use of available day-degrees (cf. Gilbert & Raworth, 1996). Using the model, it is possible to look at the parameters defining the rate increase with temperature, and compare these between populations. Tropical species have a temperature range at higher temperatures than temperate species; model parameters can be used to represent the temperature. Surveying the evolutionary possibilities of genetic variation in the model parameters leads to a deeper understanding of the evolution of temperature adaptation.

Materials and methods

The model

The Sharpe–Schoolfield model for biological rates

The Sharpe–Schoolfield equation is firmly based upon standard biophysics, i.e. upon the Eyring equation of specific temperature sensitivity of biochemical reactions (Hochachka & Somero, 1984). The Eyring equation reads:

$$r(T) = \rho \frac{T}{T_{\mathrm{ref}}} \exp\left[\frac{H_{\mathrm{A}}}{R}\left(\frac{1}{T_{\mathrm{ref}}} - \frac{1}{T}\right)\right]$$

In the Eyring equation, reaction rate, ρ(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); R is the universal gas constant (8.314 J K^{-1} mol^{-1}). The dependence of the reaction rate on temperature is given by the temperature coefficient H_{A} (in J mol^{-1}), officially the enthalpy of activation of any reaction that is catalysed by a rate-controlling enzyme. The Eyring equation describes an exponential increase of a biological rate with temperature; higher H_{A} implies a faster increase (Fig. 1A) and higher ρ implies both a higher rate at the reference temperature and a faster increase with temperature. Biological rates show this exponential increase only across a limited temperature range. Therefore, Sharpe & De Michele (1977) proposed to modify the Eyring equation. If the rate-limiting enzyme were reversibly inactivated at high and low temperature, and only fully active at intermediate temperatures, the Eyring equation can be modified by the probability that any enzyme molecule is active at a given temperature. This probability to be active is characterized by two parameters at low temperature and at high temperature: the temperature when half of the enzyme is inactive as a result of cold, T_{L} (K) or heat, T_{H}, and the specific sensitivity to cold inactivation H_{L} (the change in enthalpy associated with low-temperature inactivation of the enzyme in J mol^{-1}) or heat inactivation H_{H}. The inverse of the probability P_T for the enzyme to be active as a function of temperature is given by:

$$\frac{1}{P_T} = 1 + \exp\left[\frac{H_{\mathrm{L}}}{R}\left(\frac{1}{T_{\mathrm{L}}} - \frac{1}{T}\right)\right] + \exp\left[\frac{H_{\mathrm{H}}}{R}\left(\frac{1}{T_{\mathrm{H}}} - \frac{1}{T}\right)\right]$$

The probability of the rate-determining enzyme to be active as a function of temperature has a plateau, and decreases at both high and low temperature; higher absolute values of H_{L} and H_{H} imply a wider plateau and a faster decrease of the probability that the rate-determining enzyme is active (Fig. 1B). Incorporation of the probability of the enzyme to be active yields the Sharpe–Schoolfield equation for any biological rate as a function of temperature (Fig. 1C):

$$r(T) = \rho \frac{TP_T}{T_{\mathrm{ref}}} \exp\left[\frac{H_{\mathrm{A}}}{R}\left(\frac{1}{T_{\mathrm{ref}}} - \frac{1}{T}\right)\right]$$

The six parameters can be used in paired combinations – ρ, H_{A}, and T_{L}, H_{L}, and T_{H}, H_{H} – over specified temperature ranges of middle, low and high temperature, respectively. The Sharpe–Schoolfield model can easily be modified to account for situations where development is studied over part of the temperature range only. When development is studied over intermediate to high temperatures, the Sharpe–Schoolfield model can be modified by removing the low-temperature term in the denominator. In that case, four parameters will be estimated: H_{A} and ρ describing the temperature sensitivity of the rate over the intermediate region and H_{H} and T_{H} modifying the rate at high temperature.

The temperature dependence of any biological rate has a roughly triangular shape (Huey & Kingsolver, 1989;

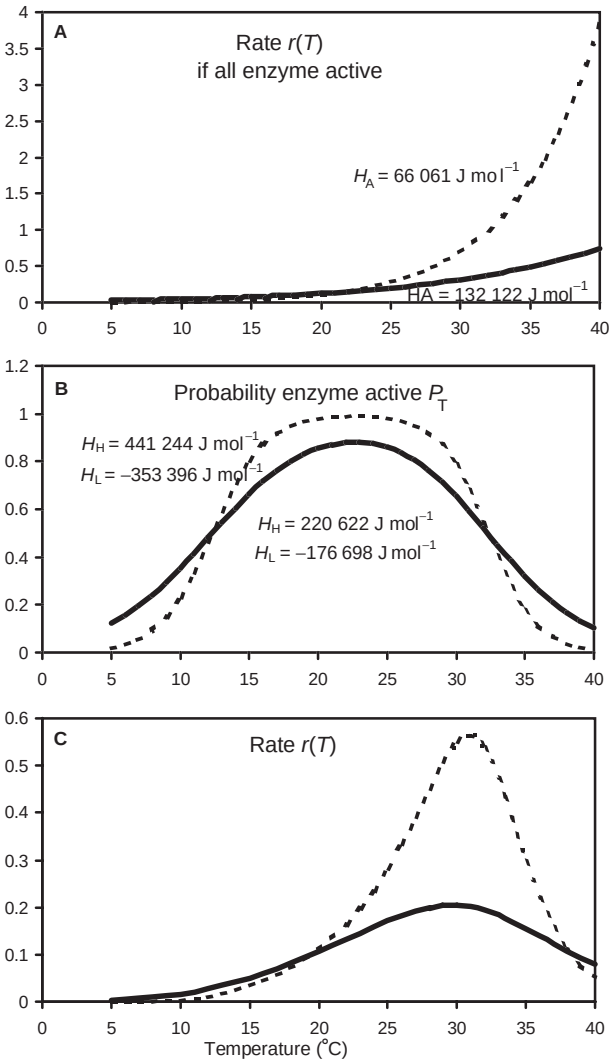

Fig. 1 The Sharpe–Schoolfield equation (C) describes a biological rate starting from the general temperature dependence of reaction rates given by the Eyring equation (A) modified by temperature inactivation of the rate-limiting enzyme (B). (A) Reaction rate pertaining to an enzyme molecule as a function of temperature; the temperature coefficient H_A differs by a factor two. (B) Reversible temperature inactivation leads to a probability for an enzyme molecule to be active. The coefficients H_H and H_L determine the steepness of the change from active to inactive; at high H_H and H_L, a plateau of full enzyme functional activity is found. (C) Combining the temperature dependence of reaction rate per enzyme with the probability that the enzyme is active leads to the Sharpe–Schoolfield equation for biological rates.

Kingsolver & Woods, 1997). The Sharpe–Schoolfield equation yields this familiar and general shape accurately (Fig. 1C). Wagner *et al*. (1984) examined the utility of the Sharpe–Schoolfield equation to describe biological rates – mostly development rate of insects – and found the model to give an extremely accurate description of development rates. Van Straalen (1994) used the Sharpe–Schoolfield equation to investigate the thermal strategies of four ecological guilds of springtails. He concluded that species living on the soil surface had a higher temperature coefficient H_A, implying higher sensitivity to temperature, but a lower growth rate ρ at the reference temperature than species living in soil. The Sharpe–Schoolfield equation proved excellently suited to ecological application.

The biophysical model for temperature dependence of adult size

Van der Have & de Jong (1996) posited that adult size can be found as the ratio of growth rate by differentiation rate. Dividing a temperature dependent growth rate by a temperature dependent differentiation rate yields a temperature dependent adult body size:

$$m(T) = \frac{\rho_G}{\rho_D}\frac{P_G}{P_D}\exp\left[\frac{H_{A,G} - H_{A,D}}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right]$$

A decrease in size with increasing temperature as observed above 18 °C in *D. melanogaster* and *D. simulans* requires the temperature coefficient H_A to be higher for differentiation rate than for growth rate. The full set of parameters of the model for adult size was only estimated for one case, using data on *D. melanogaster* published by David & Clavel (1965). As expected, $H_{A,D}$ worked out to be larger than $H_{A,G}$, representing the decline in size with temperature over the intermediate temperature range.

Species and temperatures

In this study, we used four species from different climatic zones that differ in temperature range of development. Wild living populations of the two warm adapted species, *D. ananassae* and *D. willistoni*, were collected in 1995 in India and in 1997 in Brazil, respectively. For the two cold adapted species, *D. subobscura* and *D. funebris*, wild living populations were collected in France in 1993 and 1997, respectively. All these populations were kept in bottles as laboratory mass cultures at about 20 °C until the experiments in spring and summer 1998.

The complete developmental thermal range has been described only for *D. subobscura* (Moreteau *et al*., 1997) and *D. ananassae* (Morin *et al*., 1997). The possible thermal range at constant temperature lies for *D. subobscura* between 6 and 26 °C, and for *D. ananassae* between 16 and 31 °C. *Drosophila funebris* was known to be sensitive to high temperature and *D. willistoni* to low temperature, but no detailed information was available. It turned out that for *D. willistoni* development was possible although with a high mortality at 15 °C (86% mortality), but impossible above 29 °C. For *D. funebris*, development was possible although with a high mortality at 10 °C (92% mortality) and 29 °C (98% mortality). We

used 10 incubators in this study ranging from 10 to 31 °C: 10, 12, 15, 18, 20, 24, 27.5, 29, 30 and 31 °C. The temperatures used for each species ranged from 10 to 24 °C for *D. subobscura*, from 12 to 27.5 °C for *D. funebris*, from 18 to 31 °C for *D. ananassae* and from 18 to 29 °C for *D. willistoni*.

Development time

We allowed about 15 pairs of parents to oviposit during 6 h on a killed yeast medium (David & Clavel, 1965), at 20 °C for *D. subobscura* and at 25 °C for the three other species. We transferred groups of 25 eggs into vials containing the same medium and then moved them to one of the experimental temperatures. For each species and each temperature, we used 20 replicates. We estimated development time by checking the emerging number of males and females twice a day at 9:00 and 19:00 hours (David, 1959).

Wing and thorax length

On emergence, we transferred adults to fresh medium and examined them a few days later. From each vial at each temperature, three females and three males were randomly taken to be measured. We measured wing and thorax length with a Zeiss S11 binocular microscope equipped with a LaSico automated ocular micrometer. The length of the right wing was measured from the second cross-vein to the distal tip of the wing. Thorax length was measured in a left side view, from the neck basis to the tip of the scutellum.

Data analysis

We estimated differentiation rate (D) as the inverse of the average duration of development (over the 25 flies per vial) and growth rate as the quotient of the average (over the three measured flies) wing length (G_{wing}) or thorax length (G_{thorax}) and the average duration of development from eggs to adults.

For *D. subobscura*, we estimated the parameter values of the two-parameter version of the Sharpe–Schoolfield equation by transformation. Plotting $\ln(r_T)$ on the y-axis and $1/T$ on the x-axis yields approximately a straight line with slope H_A/R and intercept $\ln(\rho)$ (Van Straalen, 1994). We estimated the parameter values of the four-parameter of the Sharpe–Schoolfield equation with multiple nonlinear regression in SAS using the Marquardt method (SAS, 1988), following a protocol by T.M. van der Have (van der Have, submitted) based upon Wagner *et al.* (1984). For all species, we used 21 °C = 294 K as the reference temperature. The 20 replicate vials at each temperature for each species were regarded as 20 replicate series of vials with identical replicate number over temperatures. Parameter value estimation was initiated for each series of vials. The parameter estimation requires convergence from random initiation values. If convergence failed, or if different initiation values did not converge on the same parameter estimate, the vial series was not taken into consideration in the further analysis.

Results

Wing size decreased with temperature in all four *Drosophila* species. Thorax size showed a maximum at intermediate temperatures in *D. funebris* and *D. ananassae* females. Development time differed between the species, but was much longer at lower temperatures. The data can be described by the four parameter version of the Sharpe–Schoolfield equation. Estimates of the four parameters H_A and ρ, and H_H and T_H, for differentiation rate and growth rate in males and females are given in Table 1. The estimates are based upon all 20 replicate series in *D. ananassae*, but in six replicate series in *D. funebris* and four replicate series in *D. willistoni* it was impossible to achieve parameter convergence. The reaction norm of differentiation rate and growth rate is almost linear for *D. subobscura* and we obtained a very bad fit with the four-parameter model for the whole intermediate to high temperature range. It was possible to calculate the four parameters for the differentiation rate in 9 of 20 replicate series, but never for the growth rate. We present estimates of H_A and ρ from a two-parameter model describing the temperature sensitivity of the rates over the intermediate region, in addition to the parameter estimates from the four parameter model for the differentiation rate. The estimates from the two-parameter model are lower, as follows from definition of the model.

Differentiation rate

Figure 2A,B shows the fit of the model to the observed data for the four species for development time and differentiation rate; the two-parameter model is used for *D. subobscura*.

We found no significant differences between sexes (ANOVA not shown), but the species differ significantly in differentiation rate. We used Bonferroni *post-hoc* t-tests to compare species two by two (Table 1). Cold-adapted species show significantly lower H_H and T_H values and higher H_A values than the warm-adapted species. H_A values of *D. ananassae* and *D. willistoni* do not differ significantly; H_A value of *D. funebris* is significantly higher than that of the two warm-adapted species. H_A value of *D. subobscura* is significantly higher than the three other species too, but this value might not be very reliable as it was based upon only nine series. ρ-Value of *D. funebris* is significantly lower that of than the two warm-adapted species.

Table 1 Average value (±SE) of each parameter calculated with a four-parameter model (Results of a Bonferroni *post-hoc t*-test are also given. Means with the same letter are not significantly different).

	Species	D		G_{thorax}		G_{wing}	
Males							
H_A (kJ mol^{-1})	*D. ananassae*	80 ± 2.2	B	83 ± 3.0	B	73 ± 2.5	B
	D. willistoni	86 ± 4.4	B	84 ± 6.1	B	75 ± 4.4	B
	D. funebris	99 ± 3.0	A	103 ± 4.5	A	95 ± 4.1	A
	D. subobscura	111 ± 6.7					
	D. subobscura	70 ± 0.4		66 ± 2.5		62 ± 0.6	
ρ	*D. ananassae*	0.338 ± 0.005	A	349 ± 6	B	484 ± 10	B
(10^{-2} h^{-1} for D,	*D. willistoni*	0.326 ± 0.013	A	285 ± 11	B	445 ± 12	C
10^{-2} cm h^{-1} for G)	*D. funebris*	0.299 ± 0.013	B	419 ± 20	A	656 ± 30	A
	D. subobscura	0.538 ± 0.069					
	D. subobscura	0.256 ± 0.0010		275 ± 2.0		409 ± 2.0	
H_H (kJ mol^{-1})	*D. ananassae*	342 ± 27	A	340 ± 22	A	343 ± 24	A
	D. willistoni	339 ± 29	A	358 ± 26	A	362 ± 30	A
	D. funebris	261 ± 20	B	285 ± 29	B	282 ± 16	B
	D. subobscura	183 ± 21					
T_H (°C)	*D. ananassae*	31.66 ± 0.28	A	30.69 ± 0.31	A	31.04 ± 0.31	A
	D. willistoni	29.13 ± 0.48	B	29.77 ± 0.51	B	29.75 ± 0.40	B
	D. funebris	26.44 ± 0.37	C	25.49 ± 0.45	C	25.74 ± 0.42	C
	D. subobscura	21.40 ± 1.05					
Females							
H_A (kJ mol^{-1})	*D. ananassae*	77 ± 1.8	B	82 ± 2.6	B	73 ± 2.0	B
	D. willistoni	86 ± 4.3	B	93 ± 5.2	B	77 ± 4.5	B
	D. funebris	97 ± 3.4	A	102 ± 4.2	A	95 ± 3.6	A
	D. subobscura	108 ± 5.2					
	D. subobscura	70 ± 0.5		67 ± 0.5		62 ± 0.6	
ρ	*D. ananassae*	0.340 ± 0.003	A	385 ± 4	B	542 ± 4	B
(10^{-2} h^{-1} for D	*D. willistoni*	0.334 ± 0.012	A	344 ± 51	B	514 ± 16	C
10^{-2} cm h^{-1} for G)	*D. funebris*	0.290 ± 0.012	B	463 ± 25	A	719 ± 31	A
	D. subobscura	0.482 ± 0.053					
	D. subobscura	0.255 ± 0.0010		309 ± 1.0		449 ± 2.0	
H_H (kJ mol^{-1})	*D. ananassae*	363 ± 29	A	355 ± 25	A	355 ± 24	A
	D. willistoni	346 ± 34	A	325 ± 30	A	390 ± 46	A
	D. funebris	267 ± 16	B	271 ± 19	B	272 ± 14	B
	D. subobscura	183 ± 16					
T_H (°C)	*D. ananassae*	31.97 ± 0.18	A	30.96 ± 0.24	A	31.28 ± 0.16	A
	D. willistoni	29.06 ± 0.47	B	28.02 ± 0.48	B	28.54 ± 0.42	B
	D. funebris	26.95 ± 0.42	C	25.56 ± 0.51	C	25.70 ± 0.39	C
	D. subobscura	22.10 ± 0.86					

For *D. subobscura* n = 9 for the four parameter model (first line) and n = 20 for the two parameter model (second line); n = 14 for *D. funebris*; n = 17 for *D. willistoni* and n = 20 for *D. ananassae*. D: Differentiation rate; G_{thorax}: growth rate for the thorax; G_{wing}: growth rate for the wing.

Growth rate

Figure 2C,D shows the fit of the four-parameter model to the observed data for *D. ananassae, D. willistoni* and *D. funebris* for growth rate of wing G_{wing} and growth rate of thorax G_{thorax}. The two-parameter model is used for *D. subobscura*. The growth rates clearly show the differences between the larger and the smaller species from a geographical region. *Drosophila ananassae* and *D. funebris*, the larger flies from the tropical and temperate regions, respectively, show the highest growth rates.

We found significant differences between sexes in only one parameter, ρ in both thorax and wing length (ANOVA not shown). Females exhibit higher values than males. Between species, we observed significant differences for G_{thorax} and G_{wing} for all parameters. We used Bonferroni *post-hoc t*-tests to compare species two by two (Table 1). The highest values for ρ, are observed in *D. funebris* and the lowest in *D. willistoni*. We found no significant differences between the two-warm adapted species in the parameters H_A and H_H. These two species

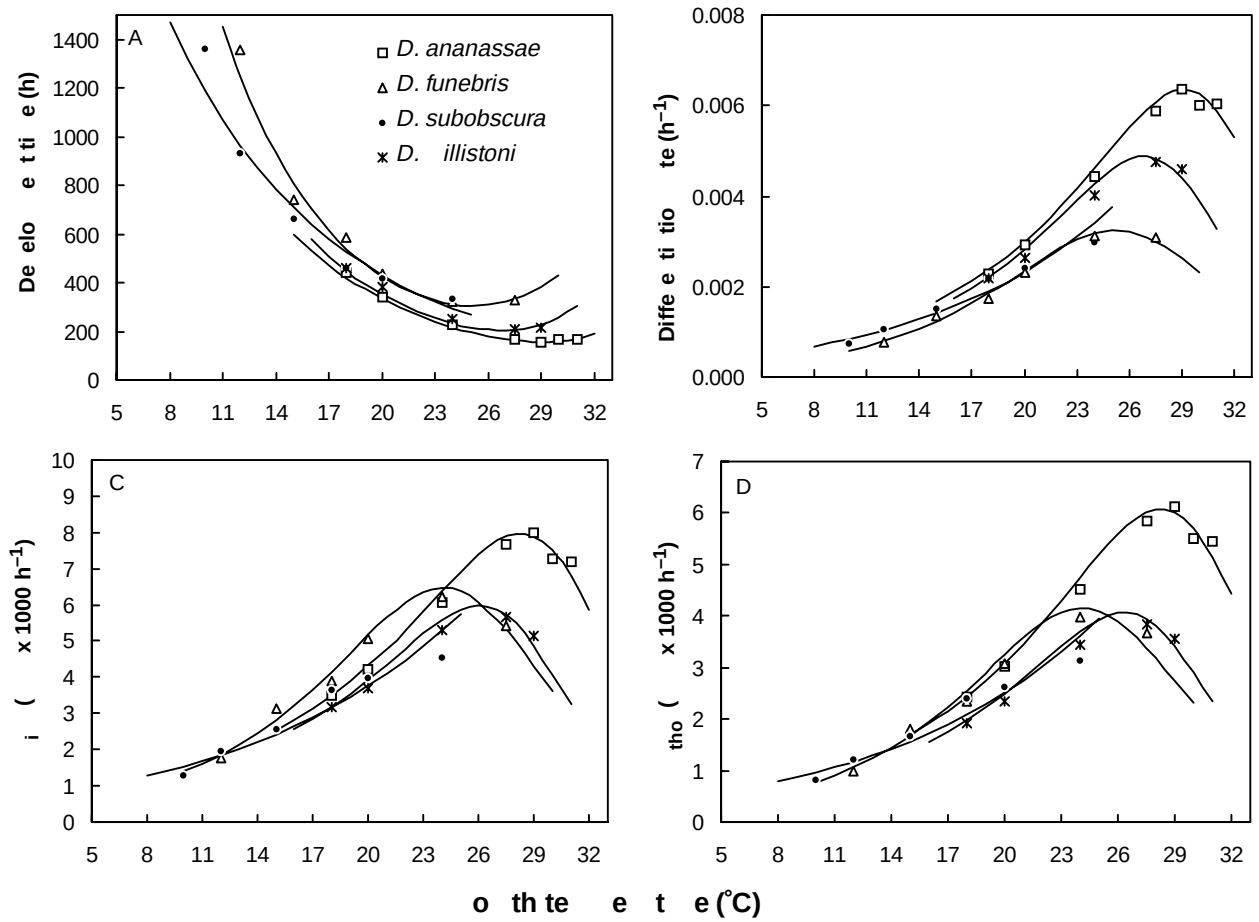

Fig. 2 Fit of the model (solid lines) to the observed data (symbols) for the four species for development time (A), differentiation rate (B), growth rate of wing G_{wing} (C) and growth rate of thorax G_{thorax} (D). The two-parameter model is used for *D. subobscura* and the four-parameter model for the three other species. The thermodynamic parameters of the Sharpe–Schoolfield equation were presented in Table 1.

exhibit higher values than *D. funebris* in H_H but lower values in H_A.

Comparison between growth rate and differentiation rate

In Fig. 3, the temperature dependence of thorax size and wing size is given; for *D. subobscura* the two-parameter model is used, for *D. ananassae*, *D. willistoni* and *D. funebris* the four-parameter model. Wing length and thorax lengths differ in their temperature pattern for *D. ananassae* and *D. funebris*. In these species wing length decreases in size but thorax length increases in size with increasing temperatures over the intermediate temperature range. The cause of this can be seen in Table 1. The temperature sensitivity of size depends upon the differences of the temperature coefficients H_A of growth rate and differentiation rate. All H_A values for G_{wing} are smaller than the H_A values for D, but the H_A values for G_{thorax} are larger than the H_A values for D for *D. ananassae* and *D. funebris* in both males and females, and in *D. willistoni* in females The difference $H_{A,G} - H_{A,D}$ is most negative for wing in the smaller species of a geographical pair, and most positive for thorax in the larger species of a geographical pair. Thorax itself is relatively temperature insensitive. The temperature sensitivity of wing/thorax ratio can be found from the difference $H_{A,G_{wing}} - H_{A,G_{thorax}}$. Wing/thorax ratio is more temperature sensitive than wing or thorax. Overall size is governed by ρ_D and ρ_G. The rank order of overall size is *D. funebris* > *D. subobscura* > *D. ananassae* > *D. willistoni* (Fig. 3). As can be seen from Table 1, the rank order in ρ differs between differentiation rate and growth rate. For differentiation rate the rank order in ρ is: *D. ananassae* > *D. willistoni* > *D. funebris*, but the growth rates of wing and thorax have a rank order: *D. funebris* > *D. ananassae* > *D. willistoni*. *Drosophila funebris* is a large species as a consequence of a high growth rate together with a low differentiation rate; *D. willistoni* is a small

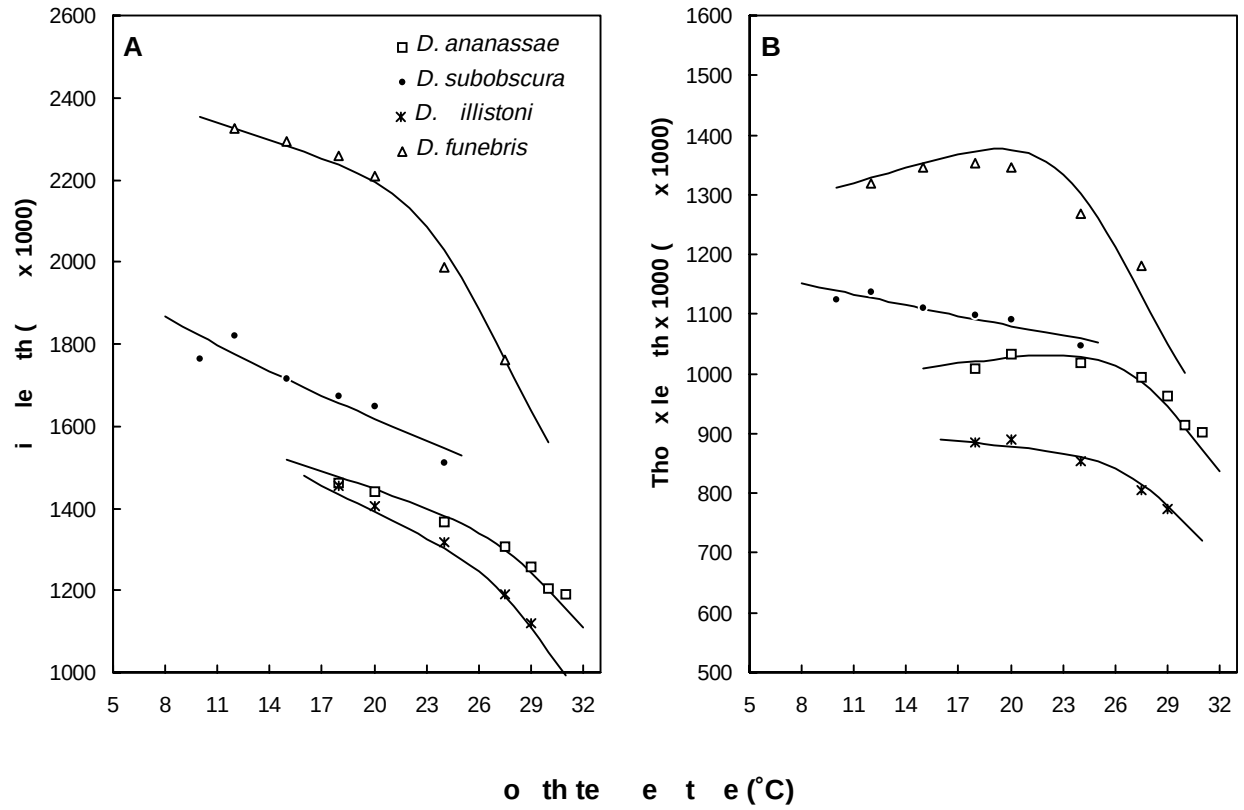

Fig. 3 Fit of the model (solid lines) to the observed data (symbols) for the four species for wing (A) and thorax (B) length. The two-parameter model is used for *D. subobscura* and the four-parameter model for the three other species.

species as a consequence of low growth rate and a high differentiation rate.

The estimates of T_H and H_H values are very similar within each species, whether calculated for D, G_{thorax} or G_{wing}. Estimates of T_H correspond to the geographical origin of the species; the highest values for T_H are observed in *D. ananassae* and the lowest in *D. funebris*. The ratio of the probabilities that the rate-limiting enzymes are active, i.e. P_G/P_D, decreases with higher temperature, causing a decrease in wing size and thorax size at high temperature in the three species *D. ananassae*, *D. willistoni* and *D. funebris* to which the four parameter model can be applied. The decrease as a result of temperature inactivation is clearly offset from any decrease in size over the intermediate temperature range. The decrease of thorax size at higher temperatures is a consequence of the declining ratio of the probabilities of enzyme inactivation, as is the additional downturn in wing size (Fig. 3).

Discussion

Many ectotherms decrease in size when raised at increasing temperatures. An adaptive explanation has proved elusive, both for the decrease and for its slope (Atkinson, 1994; Atkinson & Sibly, 1997). An adaptive explanation of body size patterns over temperature might profit from dissecting body size in its causal components. Body size in insects is widely regarded as resulting from the ratio of growth rate and differentiation rate, the inverse of development time (Ernsting & Isaaks, 1997; Nylin & Gotthard, 1998). Therefore, the temperature dependence of growth rate and of differentiation rate have to be quantified: this can be carried out by using a biophysical model of size (van der Have & de Jong, 1996).

Overall body size

The biophysical model provides interpretable biological detail on rates and size. The two tropical species *D. ananassae and D. willistoni* differ in overall size, with *D. ananassae* being larger than *D. willistoni*. The parameter estimates of the reference rates ρ_D and ρ_G for differentiation and growth, respectively, show that this size difference is a consequence of a higher rate of biomass increase in *D. ananassae*, despite the fact that *D. ananassae* has a higher rate of differentiation too. The rate of biomass increase is so much higher in *D. ananassae* that its higher rate of differentiation is more than compensated

(compare Fig. 2B with Fig. 2D). *Drosopjila ananassae* develops faster than *D. willistoni,* but gathers more biomass during this shorter development. *Drosophila willistoni* is a small species as a consequence of a relatively low growth rate (compared with *D. ananassae*) and a relatively high differentiation rate (compared with the two temperate species). The temperate species *D. funebris* is a large species as a consequence of a high growth rate ρ_G (compared with *D. subobscura*) together with a relatively low differentiation rate ρ_D (compared with the two tropical species).

Tropical vs. temperate species

The two tropical species *D. ananassae* and *D. willistoni* show higher estimates of T_H for both growth rate and differentiation rate, together with a steeper decline in enzyme activity at higher temperature as shown by a higher H_H. This is the representation in the model of the greater extent of their temperature range at higher temperatures. Moreover, the two tropical species *D. ananassae* and *D. willistoni* have higher differentiation rates than the two temperate species *D. funebris* and *D. subobscura* (Fig. 2B). A steeper increase in rates with temperature as hypothesized was not found in temperate species. The parameters give another split between tropical and temperate species, as both the ρ and H_A influence the steepness of the rate. The biophysical model in its four-parameter form shows that the higher overall differentiation rate of the two tropical species is as a result of a higher reference rate ρ at the reference temperature. The specific temperature sensitivity of the differentiation rate ($H_{A,D}$) is, however, lower in *D. ananassae* and *D. willistoni* than in *D. funebris*. This means that despite its lower overall differentiation rate, the temperate species *D. funebris* is relatively better able to increase its differentiation rate with increasing temperature. The combination of overall slow development but high temperature sensitivity of development time might represent a combination of parameters specifically suitable to adaptation to low temperature. In a seasonal environment where temperature increases in the course of the season, a high temperature coefficient of differentiation rate and development time in *D. funebris* causes the difference in time between early laid eggs and late laid eggs to be larger than the difference in time between early emerging adults and late emerging adults. A high temperature coefficient H_A therefore contributes therefore to synchronization of adult emergence (Van Straalen, 1994). Synchronization of adult emergence and subsequent improved mating possibilities have been regarded as a main factor in the selection for temperature sensitivity by Gilbert & Raworth (1996).

Clines

Geographical variation in temperature is associated with genetically different body size in *D. melanogaster* and *D. subobscura*. Some clines in body size in *Drosophila* are clearly adaptive as they are repeatable over continents (Gilchrist & Partridge, 1999; Huey *et al*., 2000). In the clines, the larger animals are found in cooler climates. Phenotypic plasticity and clines, therefore, show the same effect at the phenotypic level. However, large body size in higher latitudes is genetically based; clines and phenotypic plasticity are very different phenomena. Clines might be modelled by assuming genetic variation in the model parameters.

In our experiment, we used different species to represent different geographical regions. A tentative hypothesis might be that the geographical differentiation within a species has the same basis as the geographical differentiation between species. If so, we might expect that the more tropical populations from a body size cline within a species show higher T_H for both growth rate and differentiation rate, again together with a steeper decline in enzyme activity at higher temperature as shown by a higher H_H. More speculatively, we might expect a higher $H_{A,D}$ (i.e. steeper rate increase) for the temperate populations of the cline. This has to be worked out both experimentally and theoretically.

Temperature pattern

The biophysical model helps with the interpretation of the temperature pattern of body size. Body size in three of the four species shows a marked decline at higher temperatures, both for wing and thorax. The Sharpe–Schoolfield model of biological rates leads to the interpretation that this decline is because of inactivation of rate-limiting enzymes (i.e. would depend on T_H and H_H).

Body size at low-to-intermediate temperatures depends on ρ and H_A for growth and differentiation. Temperature independence of body size occurs when differentiation rate and growth rate have the same temperature sensitivity H_A; temperature independence of a trait is usually called temperature compensation. Body size over this range is much less temperature dependent than the underlying rates. Wing size and thorax size on the one hand, and development time or its inverse, differentiation rate, on the other hand differ much in plasticity. Differentiation rate increases some two-fold between 18 and 28 °C in *D. willistoni,* three-fold between 18 and 28 °C in *D. ananassae*, and three-fold between 13 and 23 °C in *D. funebris and D. subobscura* (Fig. 2B); but thorax size changes by 2–5%, and wing size change by 10–20% (Fig. 3A,B). The effect of temperature on development time is of a different order than the effect of temperature on body size. This is reflected in Table 1: temperature sensitivities H_A of differentiation rate and growth rate are in the same range of values (between 70 and 100 kJ mol^{-1}), but their difference is an order of magnitude lower. This difference in temperature sensitivities H_A of differentiation rate and

growth rate rules the temperature sensitivity of thorax size and wing size. Temperature sensitivity of thorax size ($H_{A,G_{thorax}} - H_{A,D}$) is between +7 and −4 kJ mol^{-1}: thorax size increases or decreases with temperature depending on the species, but is relatively insensitive to temperature and near temperature compensation. Wing size has a temperature sensitivity ($H_{A,G_{thorax}} - H_{A,D}$) between −2 and −9 kJ mol^{-1}. Temperature sensitivity of wing size is therefore consistently negative over the observed temperature range, and wing size declines with increasing temperature in the four species.

Temperature sensitivity of body size, therefore, shows two patterns over the four species: a consistent pattern of a decrease in size with temperature for wing, and a thorax size that might increase or decrease but is relatively insensitive to temperature. In the larger of two species from the same geographical origin, thorax size increases with temperature. In the smaller of the two species, thorax size decreases with temperature. The increase or decrease might be a pattern going with general size. However, thorax size might be selected towards temperature compensation to achieve maintenance of physiological performance.

If thorax size would be temperature compensated, an adaptive size decline for wing size with temperature could be argued as follows. Wing/thorax ratio is related to wing load (Pétavy, 1997), and wing load is presumably adapted to optimize flight capability at ambient temperature as predicted from the larval stage. An adaptive wing/thorax ratio would imply an adaptive and consistent difference between H_A for G_{wing} and H_A for G_{thorax}. The dependence of wing/thorax ratio with temperature is given by the difference $H_{A,G_{wing}} - H_{A,G_{thorax}}$ and is here consistently negative; the difference is about −10 kJ mol^{-1}. Thorax size might be about temperature compensated, and independently selected. If so, temperature compensation of thorax together with an adaptive wing/thorax ratio over temperatures might determine the temperature sensitivity of wing size in each species.

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