Temperature and Frog Eggs

A reconsideration of metabolic control

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ABSTRACT Bělehrádek's temperature function in which metabolic rate V is related to temperature T by

$$V = a(T - \alpha)^b$$

accurately describes embryonic development of *Rana pipiens* in different parts of its range. The constant b seems the same for all populations and a is proportional to egg diameter, at least within the United States. The scale constant α varies inversely with latitude and altitude, presumably adaptively with temperature. Bělehrádek observed that viscosity (but not reaction rates) varies similarly with temperature and concluded that biochemical rates in the intact organism are diffusion-restricted. Independent data suggest that shifts in temperature response of frog embryos and other poikilotherms may indeed be related to viscosity shifts. The fact that size (through a) may enter into Bělehrádek's function takes on more significance from Commoner's theory that species-specific metabolic rates (and cell size) may be controlled through sequestration of free nucleotides by non-template DNA. If rates are biophysically restricted they could not easily be modified by changing kinds of reactants, and control of concentration of critical reactants might be particularly effective.

Many authors have discussed the influence of temperature on development rates. This paper examines this influence more closely in a particularly suitable array of data and raises more general questions about the nature of metabolic control.

Choice of a Temperature Function

It is possible to consider metabolism-temperature curves of poikilotherms in terms of three general properties. First of all, there may be differences in the mean slope of such curves; a proportionality constant may, of course, always be extracted even from the most complex relationships. Second, there may be differences in scale position, as shown in comparisons of "warm-adapted"

and "cold-adapted" organisms. Third, there may be differences in curvilinearity. Curvature may be very complex, but generally physiological rates in poikilotherms are monotonically increasing functions of temperature within the range of temperature to which the organism is exposed in nature; experimental optima are often ecologically meaningless. To describe monotonic curves which may differ in these general properties, at least three fitted constants are required. (The "chemical" function of Arrhenius, with two significant constants, and the analogous functions of Bertholet and Van't Hoff in biology are for this reason demonstrably inadequate.) Three suitable equations, all empirically equivalent, have been used (see review in McLaren, 1963). Of these the simplest seems to be that of Bělehrádek (1935, 1957), in which rate of a metabolic function V is given by

$$V = a(T - \alpha)^b.$$

Here each constant governs a separate property: a is a constant of proportionality, α is the scale correction on the temperature T, and b may quite adequately describe the departure from linearity over the natural temperature range. Bělehrádek's function can be shown to give good descriptions of development rate, metabolic rate, and size (see examples in McLaren, 1963), but there remains the more interesting question of whether the conceptually separable constants have any separable biological meaning.

Development Rate and Egg Size

The elegant studies of Berrill (1935) revealed a simple relationship between development rate and egg size. Among thirteen ascidian genera with eggs ranging in diameter from 0.1 to 0.7 mm, but of comparable yolkiness, development time at 16°C was very nearly proportional to egg diameter. Berrill attributed this proportionality to surface/volume restrictions in gas exchange of the whole embryo. Differences in temperature adaptation, if any, were not shown in his study, perhaps because they were masked by the wide range of egg size. Nevertheless, Berrill's results pose a question: in related eggs which differ in temperature characteristics, is this relationship of development rate and egg diameter still maintained?

Development Rate of Rana pipiens

In a series of papers on frogs, beginning with that of Moore (1939), many contributions have been made to our understanding of physiological adaptation. Perhaps the most interesting of these studies deal with "thermal races" of Rana pipiens, whose eggs differ in both size and temperature characteristics but which, being closely related, might be expected to have at least some

properties in common. This material does not appear to be matched in any other group.

The available material is listed in Table I. Data from "northeastern localities" (not always specified) are from early work of Moore (1939), with additions from graphs in Moore (1942). Data from Axtla, Mexico, and all speci-

TABLE I

EGG SIZE AND THE PARAMETERS OF BĚLEHRÁDEK'S TEMPERATURE FUNCTION FITTED TO DEVELOPMENT TIME IN HOURS BETWEEN STAGE "2" AND "20" OF EMBRYOS OF *Rana pipiens*

Localities	Egg diameter	No. of experiments	Experimental temperature range	Three constants fitted			b taken as -1.75	
				a	α	b	a	α
	mm							
Northeastern* localities	1.7	30	15.3–26.0	12,740	3.4	−1.740	13,200	3.33
Vermont	1.77	25	10.3–24.1	13,450	4.3	−1.779	12,140	4.49
Wisconsin	1.76	4	10.1–22.6	13,060	3.9	−1.753	12,500	3.92
New Jersey	1.77	11	11.4–23.8	9490	4.8	−1.668	12,560	4.26
Texas	1.61	9	14.3–31.6	6140	7.0	−1.579	11,500	5.61
Louisiana	1.60	7	14.6–27.6	36,030	2.4	−2.063	11,080	4.94
Ocala, Florida	1.43	15	14.3–29.7	8740	6.2	−1.715	9910	5.94
Englewood, Florida	1.32	9	12.1–30.0	7680	6.0	−1.691	9550	5.59
Zempoala, Mexico	1.86	13	12.6–27.8	7060	6.5	−1.538	14,820	4.99
Moravia, Costa Rica	2.03	8	12.5–24.5	14,900	5.0	−1.793	12,830	5.27
Rio Tula, Mexico	1.87‡	4	12.6–25.8	31,620	3.6	−1.996	13,010	5.24
Axtla, Mexico	2.00	4	14.2–29.8	26,430	3.9	−2.005	10,190	5.90
San Diego, Mexico	1.84	9	12.6–27.8	9710	5.9	−1.720	10,750	5.72

* The experiments are partly lumped to give mean times in this work (Moore 1939, 1942) and the localities are not all specified. Some of the data may appear again in a later paper (Moore, 1949).

‡ Not given by Ruibal (1955). Taken as mean of egg diameters from three nearby localities given in this work.

fied United States localities are from Moore (1949), with a few additions from Moore (1946). Data for Rio Tula, San Diego, and Zempoala, Mexico, are from Ruibal (1955). Temperature-development data for Moravia, Costa Rica, are graphed by Volpe (1957); egg size in the same locality is given by Moore (1950).

In his earlier papers Moore (1939, 1942) compared development rate of different populations in terms of Bělehrádek's "temperature coefficient, b." In doing so he used an older version of this function, without the important

scale correction α, and therefore assumed an asymptote at 0°C. Thus an increase in scale correction could be expressed as an increase in b, which is what Moore found from north to south in *Rana pipiens*, as well as in comparisons of northern and southern species of frogs. In a later paper Moore
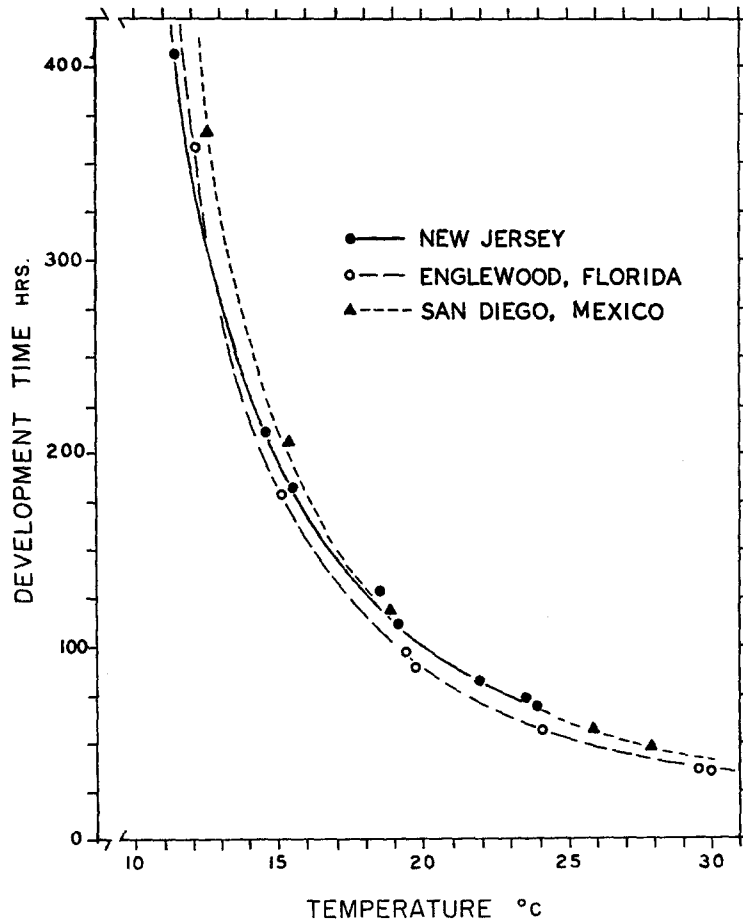

FIGURE 1. Examples of Bělehrádek's function fitted to embryonic development time of *Rana pipiens* from three localities. Mean values are shown for clarity when experimental temperatures are within 0.1°C. The fitted constants of the curves are in Table I.

(1949) abandoned this (uncorrected) function and used a graphical method of revealing differences, which was perfectly suitable for his purposes.

Information is available from thirteen localities expressing time between first cleavage (stage "2") and gill circulation (stage "20"). But before Bělehrádek's function can be applied, two essential corrections must be made. First, some of the development times are given for "20E" (early) and some for "20M" (middle, assumed to be the same as unqualified 20). From eleven

experiments where times are available for both 20E and 20 (or 20M), it is calculated that stage 20E averages 9.5 per cent earlier than 20 at any temperature. The one example given of time taken to reach "20L" (late) is reduced to 20 by the same percentage of time. Second, in many of the series severe mortality (usually accompanied by retardation) occurred at the highest experimental temperature, and sometimes at the lowest temperatures as well. All data from such extreme temperatures were excluded from consideration;
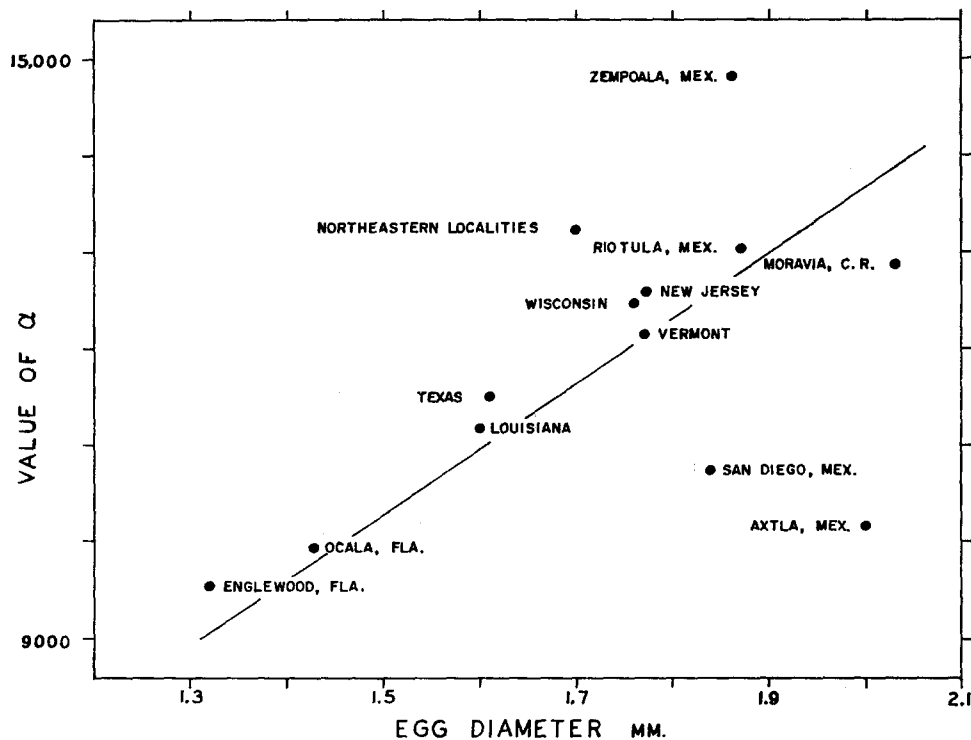

FIGURE 2. The relationship between the constant of proportionality a in Bĕlehrádek's function and egg diameter in *Rana pipiens*. The line is fitted on the assumption of proportionality to the unweighted points.

those excluded can be determined from the temperature ranges given in Table I and the original papers. Within these restrictions I have fitted Bĕlehrádek's function by conversion to logarithms and successive approximation to the nearest 0.1 °C value of α that gives smallest sum of squares of deviations. The results are in Table I and the empirical adequacy of the function is shown by representative curves, transformed to a linear scale, on Fig. 1.

When all three constants are fitted there is no support for the suggestion that a might be related to egg diameter (the correlation coefficient z for the unweighted points is 0.285, $SE_z = 0.316$). However, the fitted constants do

not appear to vary independently. It may be noted in Table I that where b deviates greatly from the mean value of the entire series of about 1.7, a also deviates greatly. If one assumes that the estimates of b are approximations to the same value in all localities, this "real" value of b may be chosen as
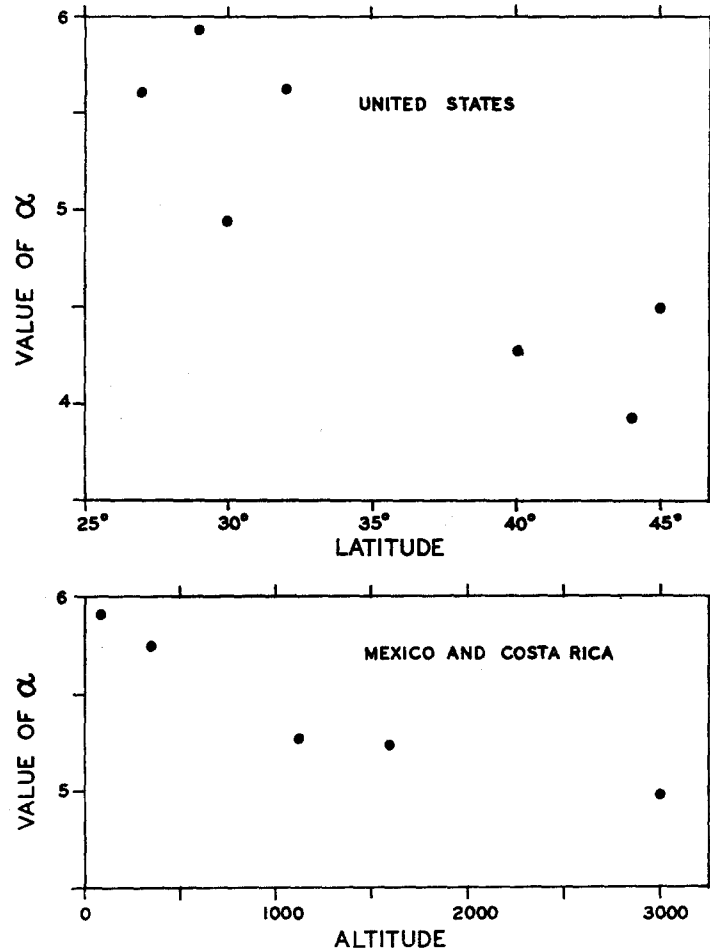

FIGURE 3. The relation between the scale correction α of Bělehrádek's function and latitude and altitude of source material.

1.75 (mean, each estimate weighted by square root of the number of determining experiments) and the other two constants fitted. There is no significant difference within any locality between the curve in which all constants are fitted and that in which b is held at 1.75. The new values of a and α are given in Table I. The correlation between a and egg diameter is greatly increased, although still just short of significant ($z = 0.582$, $\text{SE}_z = 0.316$). However, the relationship bears closer scrutiny.

The new values of a are plotted against egg diameter in Fig. 2. The line is

fitted to all the unweighted points on the assumption of proportionality. Naturally this line is even less appropriate than the least squares line (above). However, the correlation between a and egg diameter within the United States is highly significant ($z = 1.658$, $SE_z = 0.447$). Further, the origin of this relationship (at egg diameter $= 0$, $a = -14$, $SE = 108$) does not differ from zero, and allows us to assume proportionality. The addition of points from highland Mexico and highland Costa Rica does not destroy this relationship ($z = 1.224$, $SE_z = 0.354$; origin at $a = 133$, $SE = 227$). Only when San Diego and Axtla, in hot, lowland Mexico, are included does the over-all significance disappear. This could be error, considering possible methodological differences among authors and the small resolution of the underlying power functions, but it is also likely that frogs from extreme environments are not quite comparable in the pertinent physiological properties.

The other fitted constant in this function, the scale correction α, is also regularized in a logical way by assuming b to be the same in all localities (Fig. 3). Then α becomes negatively related to latitude ($z = 1.338$, $SE_z = 0.50$), and presumably positively with environmental temperature. Even within the series from Mexico and Costa Rica there is a logical correlation with altitude ($z = 1.706$, $SE_z = 0.707$).

DISCUSSION

The relationships shown here might be empirically useful. For example, to express embryonic development rate of *Rana pipiens* as a function of temperature, at least within the United States, would now require only a single experimental temperature, together with egg size. However, the results seem to raise more general and theoretical questions.

Embryonic development rate, which is defined by events in a single region or organ and in which there may be little humoral or systemic influence, may be closely dependent on the intrinsic rate of cellular metabolism. Much work on the control of cellular metabolic rate has been resolutely biochemical. Reaction rate theories have become more complex; the older collision theory (the Arrhenius equation) may be replaced by absolute rate theory (*e.g.* Johnson, Eyring, and Polissar, 1954). Efforts are also being made to construct models representing multienzyme units of metabolic activity in the cell. Such construction is to be found in the more formidable patterns of feedback control proposed by some authors (*e.g.* Chance, Garfinkel, Higgins, and Hess, 1960) as well as in the more familiar efforts of cytochemistry (*e.g.* Lehninger, 1959). To regard an egg or any cell as a black box would ultimately prove sterile, but the results given here suggest that there may be basic problems that are as readily approached by careful consideration of input-output dynamics as by analysis or reconstruction of the circuitry.

The results imply that if size is removed as a variable, then inherent differences in temperature response of closely related organisms can be described by changes in a single constant. One possible explanation of the foregoing effect is that shifts in scale position (α) may be effected by something rather general, and under relatively simple genetic control.

Bělehrádek (1935, 1957) has in fact tried to find a theoretical basis for his temperature function in observations that viscosity (but not chemical reaction rate) is affected in a comparable double-logarithmic way by temperature. Viscosity in living systems is certainly non-Newtonian, and an asymptote would be imposed at temperatures at which intracellular motion ceased, comparable to gelation in physical systems, although death would normally intervene for other reasons above this "biological zero" (α). At higher temperatures, up to the level of biochemical inactivation of the system, Bělehrádek supposed that rates would be controlled, not by biochemical reactions, but by diffusion restrictions.

There are some indications in the literature that egg properties that are possibly related to viscosity can be correlated with shifts in temperature response. For example, the work of Berrill (1935) cited earlier showed that increased yolk/cytoplasm ratio in ascidian eggs increased development time. It is especially noteworthy that Ringle (1962) has recently shown a correlation between transparency of yolk precipitates of *Rana pipiens* and development rates at the same temperatures. He feels that racial differences in temperature adaptation will be reflected in the yolk substance. Such reasoning need not be confined to embryonic development. For example, Lewis (1962) noted that chain length and saturation of fatty acids of poikilotherms may decrease with latitude and he believes that "these changes are a means of keeping protoplasmic viscosity within the range necessary for normal metabolic processes."

Finally, the fact that egg size is involved in the relationship between development rate and temperature (a in Bělehrádek's function) takes on greater significance from the recent considerations of Commoner (1964). He points out that size and metabolic rate are related to the organism's cellular DNA content, much of which has no template function. Cell size and egg size of polyploid axolotls (Fankhauser, 1952) suggest that Commoner's generalizations may validly be extended to the development of amphibian eggs. Commoner believes that the major role of non-specific DNA is in sequestration of free nucleotides that would otherwise be involved in the cell's metabolic apparatus. In this way genetically determined concentrations of critical reactants, such as nucleotides, rather than genetically controlled qualitative differences in metabolic constituents, such as enzymes, would be responsible for species-specific metabolic rates and therefore cell size. This mechanism of control might be particularly effective when reaction rates are themselves diffusion-limited.

Although the notion that reaction rates may be diffusion-limited is not entirely absent in current thinking (see *e.g.* the discussion by Ackerman, 1962), it appears that the general arguments for biophysical rather than biochemical control given by Bělehrádek (1935, 1957) have not received the attention they deserve.

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