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Introduction

Rhythms are ubiquitous in nature. In biological systems they have been well documented from bacterium [1,7,8,58], plants [5,13,14,16,29], animals [4,5] to man [9,53,62]. The periodicity spectrum of rhythms is large, since it ranges from fast oscillations (seconds, e.g. heartbeat) to years [17,23,25,56]. Even longer period oscillations do exist in some ecological systems (e.g. ~10 years hare and lynx dynamics reported by Mac Lulich in 1937 cited in [10, p. 199]) and ~ 10 years for a lepidopterous insect reported by Schwerdtfeger in 1935 and cited in [10, p. 200]). The interested reader will find some general information in the book [24] and the references cited at its end, as well as in [3,5,17,22,40,47,61,63,64].

A lot of experimental research has been, and is currently, done in this, nowadays, very active field. Besides this, rhythms, have since about a century (see e.g. Lotka-Volterra oscillating equations of population prey-predator dynamics [36,60]), stimulated research in theoretical aspects [18,20,48,57,63]. Among many simple and basic models, negative feedback has been proposed since more than 25 years ago [59] (see also [15] for an historical perspective and references cited therein). The negative feedback loop is a very common way of regulation in metabolism and in general, whose aim is to efficiently adapt supply of metabolites to organism's demand [57]. If demand is low, the concentration of the regulatory product increases and the feedback leads to an inhibition on its own synthesis. Whereas, if demand is high, the concentration of the product decrease, releasing the inhibition and thus increasing or starting a better supply. The schematic of this regulation is presented in Figure 1.

In fact, the high number of intermediates between the first (A) and last (E) substance generates a time delay between them. This results in the possibility to be out

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of phase between the actual needs of E and the regulation of A, which might ultimately result in an oscillatory system. It is indeed possible to use a simpler network by introducing directly a delay function between A and B. This approach has been developed in another paper presented in this book (see [12]). We have already proposed that such models may be of some relevance to experimental systems, since the core of some biological rhythms seems to involve this kind of regulation [2, 15, 27, 41]. In this paper we want to develop a particular aspect of biological clocks: thermocompensation. That is, the relationship between temperature and biological oscillators. Classically, one considers mainly, if not only, the effect of temperature on the period of oscillation, by the way, neglecting its action on the amplitude of the rhythmic process. Theoretical integration of temperature in a model of the per/lim circadian rhythm in Drosophila has been recently presented [33].

\[
\begin{align*}
\text{Regulation:} & \\
R_1 & \xrightarrow{\text{P}_1} A \xrightarrow{\text{P}_2} B \xrightarrow{\text{P}_3} C \xrightarrow{\text{P}_4} D \xrightarrow{\text{P}_5} E \xrightarrow{\text{P}_6} \\
\end{align*}
\]

\text{Processes (P_i)}

\text{Figure 1. The negative feedback loop network. A - E represent concentrations of intermediary substances. A is synthesized under the regulation (inhibition) of E, it is transformed to B, then B to C, etc..., according to different processes P_i's (enzymatic, transports, etc...).}

Temperature interaction with biological clocks is rather puzzling. If one considers the "classical" view: circadian rhythms (~24h period) are thermocompensated (i.e. their period is independent of T), whereas for ultradian rhythms (period < 24h) the period is temperature dependent [e.g. 39]. This view is clearly challenged by clear experimental evidences that show that some ultradian rhythms may be thermocompensated [26, 30, 31], and some circadian rhythms may be not [6, 38, 44, 46, 58]. However, it is relevant to say that these latter situations seems to be exceptions. We will be interested in this paper to circadian rhythms mainly. The whole picture of thermocompensation in this case is rather intriguing for the following reasons:

1. Elementary rate reactions are always accelerated by temperature, and metabolism in general shows the well-known relationship:

\[ Q_{10} = \frac{\text{rate}_{T+10}}{\text{rate}_T} \geq 2.0 \]  

This means that for an increase of 10°C, we do have a doubling of the observed rates [e.g. 47].

2. But, the period of thermocompensated rhythms has a \( Q_{10} \equiv 1.0 \). This means that the frequency/period doesn't vary with temperature (in physiological ranges) [5, 17, 47].
3. The amplitude of the rhythm may show contrasting results. It could either be inversely, not or dependent on temperature (i.e. $Q_{10}$ is between 0.5 - 2.0). In fact, very little attention has been paid to this aspect in the literature [17,47].

4. However, the rhythm, although temperature independent, can be synchronized (phased) by environmentally cycling temperatures [5,17,47].

5. From the previous property (point 4) it follows that the rhythm possess a typical phase response curve (PRC) to temperature. This means that depending on the relative internal time of the oscillator, a step or pulse change in temperature, may lead during the stimulus cycle or next one, to either an advance (shortening of the period), or no effect, or a delay (lengthening of the period). A typical PRC is shown in Figure 2.

![Figure 2. Phase response curve of a pulse of 8°C on the circadian leaf movement of *Phaseolus*. 0 time is with respect of the minimal (downside) position of the leaf rhythm. The period of the control rhythm (20°C) is 28.4 h. The duration of the pulse is 4 h. (+) shift of the period of the cycle during the pulse. (<>-) shift of the period on the cycle following the treated one. (->-) shift of the period of the next cycle. Data are adapted from Moser [43].](image)

It is relevant to observe in this figure that the effect of the 4 h temperature pulse disappears during the second cycle after the treatment.

Current research is in progress to understand at the molecular/genetic level the mechanisms underlying temperature compensation in biological rhythms [28,35,37,42,49,52].
Achieving thermocompensation in a rhythmic model with a negative feedback loop regulation.

The schematic representation of a negative feedback network is presented in Figure 1. The corresponding model, in its Michaelis-Menten formulation (for more details see [15]) is written below (equations 2 to 6):

\[
\begin{align*}
\frac{dA}{dt} &= \frac{v_0}{K_s^4 + E^4} \frac{V_m A}{K_m A + A} \\
\frac{dB}{dt} &= \frac{V_m A}{K_m A + A} \frac{V_m B}{K_m B + B} \\
\frac{dC}{dt} &= \frac{V_m B}{K_m B + B} \frac{V_m C}{K_m C + C} \\
\frac{dD}{dt} &= \frac{V_m C}{K_m C + C} \frac{V_m D}{K_m D + D} \\
\frac{dE}{dt} &= \frac{V_m D}{K_m D + D} \frac{V_m E}{K_m E + E} 
\end{align*}
\]

These equations describe the dynamics of the system in terms of rate changes of its state variables (concentrations of A – E). \( v_0 \) is the constant synthesis flux of A modulated by an inhibitory function (depending on E). \( K_s \) is the constant of inhibiting function with a cooperativity factor of 4. \( V_m \) and \( K_m \) are the michaelian constants respectively, the maximum enzyme velocities and affinities for substrate (see also Figures 3 and 4 for an explanation of these terms and [15]).

![Figure 3. Illustration of the effect of temperature on processes (enzyme reactions following Michaelis-Menten kinetics). A rise in 10°C temperature doubles the Vmax (\( Q_{10} \) Vmax = 2.0 and doubles the affinity (\( Q_{10} \) Km = 0.5, i.e. the Km value decreases by a factor of 2.0).](image)
The dependence of this model to temperature may be introduced by a similar way as already described in [11]. In brief, Vm's have they value increasing following an Arrhenius law and a $Q_{10}$ of 2.0. Km's have their values decreasing with an Arrhenius law, with a $Q_{10}$ of 0.5. This is normal since it is mainly expected that increasing temperature will increase the affinity. However, *increasing* the affinity means *decreasing* the Km value. At this step, a simulation will yield to an ordinary non-temperature compensated oscillatory system (the simulation will be presented later). To obtain the effect one can manipulate individual Vm's and Km's till obtaining the desired effect. A very elegant method has been proposed by Ruoff *et al.* [51] and Ruoff [50]. They let the computer change randomly (within given constraints) the values till by this artificial selection method, solutions where thermocompensation occurred were found. With this method they obtained the wanted effect, with more than one solution. In our case, we do want to obtain by the simplest and direct way thermocompensation. After some preliminary trials we rapidly found that changing the dependence of $K_{IE}$ towards temperature was the key solution. Indeed, manipulating Vm's is rather difficult and limited since they necessarily will be in each case increased by temperature, whereas for $K_{IE}$ and Km's, it may happen that temperature could lead to the inverse expected result (justification of this will follow). In practical a solution of thermocompensation (other solutions may likely be found, but we don't have checked them yet), has been obtained by assuming that each Vm's is temperature dependent (with a $Q_{10}$ of 2.0), all Km's are also temperature dependent in the expected direction ($Q_{10}$ of 0.5, i.e. increasing the affinity by a factor of 2 with a 10°C interval). The only requirement was a modification of $K_{IE}$, where the affinity of the inhibitor will decrease at higher temperatures ($Q_{10} = 1.5$). Figures 3 and 4 give a graphical representation of the meaning of all these numbers.

![Figure 4. Illustration of the effect of temperature on a regulation system (inhibition, cooperative 4th order). An increase of 10°C decreases the affinity of the inhibitor by 1.5 ($Q_{10} K_{IE} = 1.5$).](image-url)
Justification of the approach

It is necessary to justify the mentioned approach, since it may appear counterintuitive that an affinity (K_{iE} or, if necessary, K_{m,i}'s) for an inhibitor (or a substrate) could decrease instead of increase with rising temperatures.

A. Theoretical arguments

One needs to reconsider the Michaelis-Menten kinetics for enzymes, this kinetics may be expressed in terms of elementary rate reactions (which themselves are always accelerated with an increase in temperature, see Figure 5).

\[
E + S \xrightarrow{k_1} ES \xrightarrow{k_2} E + P
\]

Figure 5. Michaelis-Menten reaction kinetics for enzyme catalysis with a single substrate (S) and product (P). E is the enzyme, ES the enzyme-substrate complex and the k's are the kinetic constants of the reactions.

E is the enzyme, S the substrate, ES the enzyme-substrate complex and P the product concentration's. k's are the constants of the elementary rate reactions. The rate of the reaction (destruction of substrate S or appearance of the product P), is given by the well-known Michaelis-Menten equation:

\[
\frac{dS}{dt} = \nu = \frac{V_{\text{max}} S}{K_m + S}
\]

(7)

The temperature effect on the different parameters are as follows:

1. On the elementary rate reactions, the effect will follows the Arrhenius law such as:

\[
k_i(T) = A_i e^{\frac{E_i}{RT}} = A_i e^{\frac{E_i}{T}}
\]

Where R is the gas constant, T the absolute temperature, E_i, the activation energy and A_i, the frequency factor (see [32]). The extent to which each parameter depends on temperature depends on these 2 factors. These will define more or less rapidly starting and increasing dependencies.

2. On the V_{\text{max}}, actually this parameter represents:

\[
V_{\text{max}} = k_3 S
\]

(9)

This means that the effect of temperature will be similar as for the elementary rate reactions, i.e. it should always somehow increase in principle.

3. On the K_m:

\[
K_m = \frac{k_2 + k_3}{k_1}
\]

(10)

It is clear that K_m being a ratio, it is a non linear function of the elementary rate constants. Should the positive dependency of k_1 towards the temperature being smaller than for k_2 + k_3 then K_m will increase with temperature instead of
decreasing. In other words, in this situation the affinity will decrease at higher temperatures. Actually Km may decrease, be independent or decrease according to this factor and these theoretical considerations.

B. Experimental arguments

It is clear that such considerations, being purely theoretical, may be only hypothetical. However, experimental dependencies have been measured for Km at different temperatures. These values have been measured for phosphoenolpyruvate as substrate for the enzyme pyruvate kinases in different species by Somero & Low [55]. These important results are presented in Figure 6. As it might be observed for the same enzyme from different species the apparent Km may be either decreasing (expected situation), or more or less independent or, finally, increasing with higher temperatures. In this latter case the affinity decreases with increasing temperatures. The experimental observations match very well the theoretical considerations and give a physicochemical explanation for them. In fact, even temperature compensated chemical reactions have been found as early as in 1915 [54]. Winfree [63] advocates for a very common occurrence of temperature compensated phenomena's. Other experimental evidences of temperature compensated Km can also be found in [45]. It is therefore not unrealistic for K_i, which is an apparent inhibitory constant, whose relationship with elementary rate reactions might well be also non linear, to hypothesize an inverse dependency on temperature.

![Figure 6. Temperature effect during the assays on the apparent Michaelis constant (Km) of phosphoenolpyruvate for pyruvate kinases from different species. Data is adapted from Somero & Low [55].](image)
Figure 7. Simulation of the negative feedback loop model according to equations (2) to (6). All temperature dependencies of the parameters increase as expected with temperature (i.e. $Q_{10} V_m's = 2.0$, $Q_{10} K_m's = 0.5$ and $Q_{10} K_1 = 0.5$). The simulation shows an oscillation with a period accelerated by 2 (i.e. $Q_{10}$ period $= 2.0$). There is no temperature-compensation in such a system.

Figure 8. Simulation of the negative feedback loop model according to equations (2) to (6). All temperature dependencies of the parameters increase as expected with temperature (i.e. $Q_{10} V_m's = 2.0$, $Q_{10} K_m's = 0.5$) excepted for $K_1$ where the affinity will decrease with temperature (i.e. $Q_{10} K_1 = 1.5$). The simulation is now temperature-compensated (compare with Fig. 7).
Simulation of the temperature-compensated negative feedback loop

Dynamics of the system can be obtained after numerical integration of equations (2) to (6) with a numerical method (Runge-Kutta 4th order). Figure 7 shows the results of inserting the temperature dependency into the different factors ($V_m$, $K_m$, $K_{IE}$) on the simulation of the model. All the temperature parameters dependencies have been chosen to have a $Q_{10}$ of 2.0 (control). In this case we obtain after simulating a rise of 10°C, that the system oscillates with a period that is half of that before the temperature rise (i.e. a doubling of the frequency). This means that the timing mechanism is not temperature-compensated and, more importantly, that the system period has a $Q_{10}$ of 2.0, which is a result not unrealistic. The amplitudes are always reduced, but depending on the intermediate considered, with different proportions. In Figure 8, we show the same simulation, but in this case the $K_{IE}$ dependence on temperature has been

![Figure 9](image_url)

Figure 9. Effect of cycling temperatures (amplitude 10°C) on the negative feedback loop temperature-compensated model. Upper graph: control with no cycling temperature. Middle graph: cycling at a lower frequency than the endogenous control period. Lower graph: synchronization by a frequency higher than the endogenous control period. In all cases the rhythm, although temperature-compensated, is clearly entrained by external temperature cycles.
inverted as already discussed. In this case the period is thermocompensated (i.e. the $Q_{10}$ of the period is 1.0). One might observe a very interesting result: the amplitude of intermediate A is, this time, increased, whereas for E it remains almost unchanged. This happens within the same simulation! This observation might explain why experimental temperature effects on amplitude have received so little attention and might present apparent contradictory results in literature (see point 3 in the present introduction). All this means that the timing is independent of temperature, whereas this factor might have very different actions on amplitude, depending on which state variable one is actually observing in the system.

Given that the period of this model rhythm is temperature-compensated, could it nevertheless be that the rhythm is entrained by temperature cycles (point 4 in introduction)? The answer to this question is given in Figure 9 and is definitely positive.

One might thus expect that the temperature-compensated model rhythm will possess a typical PRC towards temperature pulses. This is indeed the case, as it could be observed in Figure 10. Not only is the functional similarity with Figure 6 nicely surprising, but even more surprising are others details like, for example, the fact that in the simulated rhythm the equilibrium period is attained after the second cycle (compare with Figure 6). Also to note is that the simulation model has its first cycle more affected than its second one. It is noteworthy to mention that all these results were quite unexpected given that the small change to achieve thermocompensation was very simple a priori (from $Q_{10} K_{IR} = 0.5$ to $Q_{10} K_{IR} = 1.5$).

![Figure 10. Phase response curve (PRC) of the negative feedback loop temperature-compensated model. A pulse of 10°C has been inserted at different times in the basic cycle (its period length has been set arbitrarily to 1 a.u.t.). Time 0 is the minimum of A. The duration of the pulse is of 1/6 a.u.t. (i.e. 4 h in a cycle of 24 h). Depending when the pulse is given, the effect on the 1st cycle (i.e. the cycle during which the pulse is given) shows either lengthening of the period (delay) or shortening (advance). Effects are also observed with a lower amplitude on the next untreated cycle (2nd cycle). Temperature compensation is observed at the 3rd cycle. Compare with Fig. 6.](image)
Thus, once it is possible to artificially obtain a model temperature-compensated rhythm, all other intriguing experimentally observed features (points 2-4 in the present introduction) are simultaneously observed without any implementation in the model.

**Conclusion**

More realistic, yet also considered minimal, models have been presented to simulate circadian clocks recently [19,20,21,34]. We have presented here a more general and abstract model based only on the negative feedback regulation. Indeed, it is always possible to modify the model, in order to include new parameters/functions/variables according to new experimental discoveries. However, the core of the oscillating mechanism, in this situation, will remain unchanged. That is, oscillations will arise in negative feedback loops whenever particular time relationships (delay) between the regulator and its target are met.

It is a matter of fact that it is always possible (and yet easy) to argue that such a model has nothing to do with reality. Although this point of view could be in fact very seriously challenged, we should ask then what could be the essential interpretation of such simulations? Actually modeling should be considered as a complementary tool to investigate systems, this method might provide some new insights into the functioning of the system or it might also provide some new experimental testing or working hypothesis. It is therefore a complementary tool which should not be simply disregarded, just by lack of understanding, or, on the counterpart, by its apparent simplicity.

In the present situation one has to look to the lessons these simulations might bring, in order to eventually implement future experiments. The whole story of results presented here simply suggests that:

1) Temperature compensation might be achieved in a complex network (like a biological rhythm) by just controlling one very specific step at a very basic molecular/physico-chemical level, so that the whole network is completely temperature regulated. Alternatively, there are no theoretical exclusions for multiple targets for temperature compensation (see [50,51]). Overall, this means that temperature compensation mechanisms in rhythms might be very diverse and could have been achieved in evolution by very different ways. This is also a point of view similar to the one already presented by Winfree [63].

2) Once temperature compensation has been achieved by such a way, there is no necessity to imagine other mechanisms to explain the other intriguing experimental observations done with this environmental factor, because they are a direct consequence of the temperature compensation mechanism itself.

3) The effect of temperature on amplitude may vary within the same rhythms. Depending on the variable examined one may obtain a decrease, no effect or an increase in this parameter.

4) Last, but not least, it is extremely interesting to observe that thermocompensation may be achieved by respecting classical physico-chemical laws (i.e temperature always increase elementary rate reactions kinetics). It seems like, at the moment, it is not necessary to imagine new particular mechanisms at the physico-chemical
level. However, microstructures (e.g. proteins, enzymes and their catalytic properties) characteristic to life have themselves complex relationships (non-linear) with these elementary physico-chemical laws in such a way that new properties may now emerge. In other words, from a basic monotonous temperature dependency (increase only) for elementary rate reactions, the integration by living systems (e.g. enzymes) may now lead to dependencies now far more complex (increase, independent, decrease). The evolutionary pressure may then work to "adapt/fit" the available larger potential dependency "coherently" with the environment/organism's needs.

Testing experimentally these interpretations could be a rather challenging and exciting perspective.

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References

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60. Volterra V (1931) Théorie mathématique de la lutte pour la vie. Gauthier-Villars, Paris


64. Young MW (1993) Molecular Genetics of Biological Rhythms, Marcel Dekker, Inc New York