Pittendrigh: The Darwinian Clock-Watcher

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Vijay Kumar Sharma's major research interests presently are circadian organization in fruit flies, ants and mice, the adaptive significance of circadian rhythms, role of these rhythms in development and aging, molecular basis and ontogeny of circadian rhythms and the role of circadian clocks in psychiatric disorders. His earlier papers on the light relations of the circadian rhythms in the field mouse were directly inspired by some of the postulates of Colin Pittendrigh.

For Glossary, see p.41.

This essay is an expression of my long-standing desire to illustrate the exceptional and voluminous research contributions of one of the founding fathers of Chronobiology, Colin S Pittendrigh, for present and future students of the field. I have no doubt that it is not going to be an easy task to write a summary of Pittendrigh's contributions, but I will try and provide the readers with some glimpses of his research which helped build the foundation of an entire new discipline which we call Chronobiology. Many of the contributors to this issue of *Resonance* knew Pittendrigh personally, as collaborators contemporaries and students, but I am writing this article as a "second generation student", one who learnt the tricks of the trade by reading his papers. Pittendrigh's papers carry insightful concepts, creative ideas and plausible hypotheses, many of which have not yet been tested. They describe lines of research, which address fundamental scientific questions some of which have been abandoned due to practical difficulties that arose at the time they were carried out. Modern techniques could make them feasible, yet, there have been very few attempts to revive those lines of research (see also Carl Johnson's article in this issue). I believe that revisiting some of the issues that are buried in his papers is guaranteed to contribute a wealth of information to our current understanding of how timing systems work in living organisms. In my personal opinion some of Pittendrigh's papers should be read by every student who wants to build a career in chronobiology.

Keywords
Pittendrigh, circadian rhythm, phase-response curve, temperature compensation, evolutionary processes.

Not very long ago chronobiology was considered a mysterious subject, and the very mention of "biological clock" would evoke a sense of skepticism. Today, it is considered as a mainstream scientific discipline. There is almost no issue of Science, Nature, Cell and Neuron Magazines without at least one paper on this topic. Every field must have its own defining moment; an event to remember as the moment of its birth. For Chronobiology it was the Cold Spring Harbor Symposium on Quantitative Biology, a meeting which was organized to commemorate the memory of Gustav Kramer. The scientific presentations of this meeting were chronicled in a volume titled the Cold Spring Harbor Symposia on Quantitative Biology, Volume XXV, 1960 (hence forth referred to as the *proceedings*). It is fun to read the *proceedings* and compare the status of the science, and approach, between then (1960) and now (2006). One obvious and expected difference is in the use of many modern genetic and molecular tools, which have indeed helped us in understanding the intricate details of many complex mechanisms underlying circadian time keeping. A fact that immediately catches one's attention is the enormous number of species investigated previously compared to the current reliance on about a dozen model organisms e.g., human, rat, mouse, hamster, chicken pineal gland (*in vitro*), frog, zebrafish, fruit fly, fungus, plant, and cyanobacteria. The abysmal numbers of plant and protists in particular is a reminder that current chronobiology research is very animal-centered, primarily due to the pressure to use models that can be functionally linked to the analyses of human disease. Back in the 1960s when nothing was known about clock genes and the underlying molecular mechanisms, some fairly plausible models were put forward, that are not too different from what the current models hypothesize. It comes as a big surprise to the current researcher in the field to read the *proceedings* and realize that practically everything we know now was already anticipated as early as 1960. For example, look at the impressive list of 'empirical generalizations' about biological clocks made by Colin Pittendrigh (probably the most cited paper in the field, ever). We have added detail, lots of detail, but no new concepts, no new rules, and few new creative experimental protocols.

Well, we have come full circle. After three and half decades of extensive research in modern biology (molecular biology) starting with the discovery of period (*per*) gene in *Drosophila* by Konopka and Benzer in 1971, the localization of suprachiasmatic nucleus (SCN) to the description of interlocking feedback loops in plants and animals, we are now talking about research which would endow functional value to the circadian organization. For instance there is greater appreciation now for questions related to the relevance of circadian rhythms in the context of behaviour, neural plasticity, physiology, sleep, navigation, sociobiology, migration, hibernation, life history, and adaptation – issues that were discussed long ago by Pittendrigh and his contemporaries. Pittendrigh devoted his entire research career to providing us with a whole gamut of ideas without invoking specific genes or molecules. He did not believe that only a handful of genes are sufficient to run circadian clocks, a view which many of us in the field have begun to realize only now. We can clearly see from Pittendrigh's papers that he was always fascinated by the functional values of biological clocks. This view dominated and guided his research to a great extent. His evolutionary approach to studying the behavioral and physiological mechanisms underlying biological clocks brought tremendous value to his scientific papers published between 1954 to 1993. These papers provide us with some revealing glimpses into his scientific philosophy and his remarkable persona.

Pittendrigh's research findings not only served as a motivating factor for a large number of researchers all around the world but also formed the basis of a rapidly emerging discipline that is practiced at basic and applied levels throughout the world. It contributed towards building the genetic, physiological, evolutionary and ecological framework for research in chronobiology in a wide variety of organisms.

A large number of biological processes, both simple and complex, are oscillatory in nature, occurring with a periodicity of 24 h, giving it a likeness to the periodicity of the environmental geophysical cycles. Although some of these rhythmic processes may be simply mimicking environmental changes, many are expressions of endogenous mechanisms, believed to be an outcome of millions of years of interaction between the biological and the geophysical world. When organisms are isolated from the influence of periodic environmental factors – by maintaining them under constant laboratory conditions with light, temperature, humidity and sound kept constant - a large majority of biological rhythms display a near 24 h free-running period pattern (*circa* = approximately, *dies* = day hence circadian rhythms) (*Figure* 1). Pittendrigh's extensive work on the adult emergence (eclosion) rhythm has greatly contributed to our understanding of the basic nature of the circadian clocks in *Drosophila* and

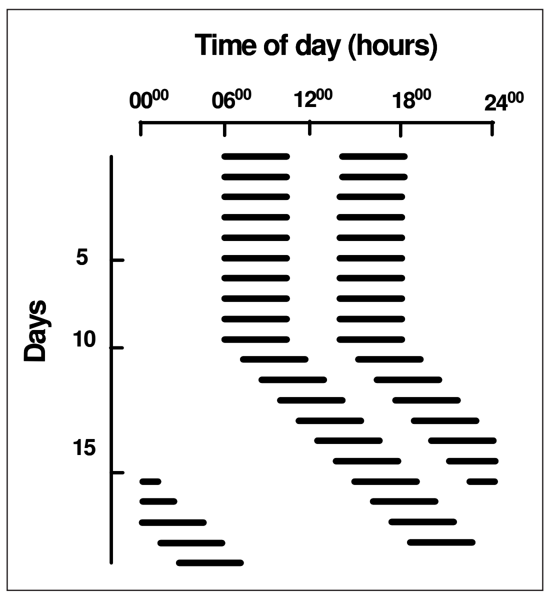

Figure 1. Schematic representation of activity/rest cycles of an animal in presence (first ten days) and in absence (last ten days) of light/dark cycles. Time of day is plotted along the abscissa and number of days along the ordinate. Activity/rest data is arranged one below the other chronologically to facilitate easier visualization of activity patterns. Presence of dark bars represents activity and its absence represents rest. The activity/rest rhythm of the animal entrained (synchronized) to 24 h light/dark cycles, i.e. its period matched that of the light/dark cycle and its phase adopts a stable relationship with the light/dark cycle. The activity/rest rhythm starts free-running with a near-24 h (circadian) period as soon as it is transferred into constant darkness.

its entrainment by light/dark and temperature cycles. In a span of about 15 years he published a numbers of papers describing the evolutionary and basic physiological processes and providing empirical generalizations of eclosion rhythm in *Drosophila*. Pittendrigh's early research quite convincingly demonstrated that the circadian oscillation in adult emergence behaviour in *Drosophila* is a gated phenomenon, completely independent of the rate and the stage of morphogenesis and differentiation. He discovered that circadian clocks gate the act of emergence of adults from pupae to a restricted phase of the oscillation; and since the oscillation itself locks on to the light cycle in nature, it gates adult emergence to a limited time of day. The gating is so stringent that if adults are mature enough to emerge but fail to do so during the gate, they would need to remain within the puparium until the next gate opens. Therefore populations of pupae that manifest a rhythm of emergence activity consist of individuals, which may not be synchronous developmentally, but are fully synchronous in their circadian oscillations. Pittendrigh realized that such timing systems would be less useful if they were temperature dependent. Imagine a biological time keeping device that is based on metabolic processes that speed up with increase in temperature and slows down with decrease in temperature. The clock will leave the owner confused as it will run faster at high temperatures and slower at low temperatures. As homeotherms we may be safe to a certain extent, but imagine the fate of poikilotherms such as fruit flies. It is obvious that a temperature-dependent clock will not only guarantee mistiming but would measure time differently during summer and winter, during day and night, and therefore would be unreliable. In 1954 Pittendrigh showed that although the development of fruit flies was largely dependent upon temperature and would speed up during summer and slow down during winter, the timing between two successive peaks of eclosion are temperature compensated, and do not change much with increase or decrease in temperature. This suggests that circadian clocks are capable of compensating for changes in environmental temperature. Thus, unlikely as it may seem on physical grounds, the biological prerequisite of temperature compensation of clocks has been achieved even by poikilotherms.

Pittendrigh strongly believed that circadian timing systems have evolved through adaptation to periodic factors in the geophysical environment. He postulated that the daily light/dark cycle was the primary force behind the emergence and subsequent maintenance of circadian clocks. He proposed that circadian clocks have evolved as an evolutionary consequence of efforts to avoid deleterious effects of light. Since a number of cellular functions are affected by light, he speculated that organisms may have restricted some of their metabolic processes to the night phase to avoid adverse effects of light. As early as 1960 Pittendrigh performed the first laboratory selection experiments on *D. pseudoobscura* and *Pectinophora gossypiella* and derived strains (the *early* and the *late* strains) that emerged in the "morning" and the "evening" hours. The peaks of adult emergence of the *early* and the *late* strains were separate from each other by about 4-5 h, after fifty generations in *D. pseudoobscura*, and after nine generations in *P. gossypiella*.

In 1930s Erwin Bünning, whose work profoundly influenced Pittendrigh, proposed the "coincidence" model for the causal relationship between circadian rhythmicity and photoperiodic time measurement. According to this model photoperiodic induction occurs in organisms only when a specific ("inducible") phase-point in the organism's circadian clock coincides with an appropriate phase in the environmental light/dark cycle. On the other hand Pittendrigh believed that light/dark cycles have dual roles in photoperiodic time measurement: (i) it entrains (synchronizes) circadian rhythms, thus establishing a determinate phase relationship between the rhythm and the light/dark cycle, and (ii) it causes photoperiodic induction. Given that the above two functions of circadian clocks were quite distinct he suggested that two separate photoreceptor pigments may be involved in entrainment and photoperiodic induction. Between 1970 and 1980 Pittendrigh worked extensively towards understanding of entrainment mechanism and its role in photoperiodic time measurement. He believed that the key to understanding photoperiodic time measurement lies in our ability to understand circadian entrainment sufficiently well, so as to be able to predict as accurately as possible which light/dark cycles-natural or contrived-would cause photoperiodic induction, and why. His studies demonstrated that organisms have the ability to track photoperiodic changes in their environment by maintaining a stable, reproducible phase-relationship with the light/dark cycles.

Pittendrigh proposed a dual oscillator model to account for the behavioural features of circadian eclosion rhythm of *Drosophila*. According to this model, one of the oscillators, the master oscillator, was assumed to be light sensitive and temperature compensated while the other, the slave oscillator was assumed to be refractory to light and temperature sensitive. Light and temperature perturbations are often followed by several transient cycles. In a serious of carefully designed experiments he perturbed the free-running eclosion rhythm of *Drosophila* using light and temperature stimuli. He demonstrated that light induced transients do not represent the state of the master oscillator, and that in fact the transient cycles in the eclosion rhythm of *Drosophila* were the overt expressions of the light insensitive slave-oscillator, gradually regaining its phase relationship with its master.

Pittendrigh also held the view that a multicellular organism hosts a large number of cyclic metabolic processes that can be likened to a population of circadian oscillations. For the normal functioning of organisms under periodic light/dark cycles, these oscillators must to be coupled to each other in such a manner as to have stable phase and period coordination.

Pittendrigh believed that there would be dire physiological consequences if the internal temporal order among constituent oscillators is disrupted. He proposed the “circadian resonance hypothesis” according to which organisms with circadian clocks whose periodicities matched that of the environment they lived in, would perform “better” compared to those whose periodicities do not match the environmental cycles. Using *Drosophila* populations he demonstrated that flies lived significantly longer under 24 h light/dark cycles than under light/dark cycles of 21 h, or 27 h or under continuous light environment.

Pittendrigh’s work during the first half of 1970s involved characterization of circadian entrainment, stability, homeostasis, and entrainment in nocturnal rodents under a wide range of environmental conditions. His studies showed that the free-running circadian activity rhythm of golden hamsters and two species of deer mouse became progressively shorter as the animals became older, suggesting that circadian clocks age like their owners. In subsequent studies he showed that the age-related period shortening in rodents was due to an increase in testosterone levels in adult mice. Castration of mice free-running under constant darkness caused an increase in the free-running period of the activity rhythm and subsequent implantation of Silastic capsules from which a physiological dose of testosterone was released at constant rate restored the animals clock to its old configuration.

Pittendrigh was intrigued and challenged by the issue of entrainment through out his career. He worked out a non-parametric entrainment model for eclosion rhythm in *Drosophila*. The essential elements of this model are the two key properties of the circadian pacemaker: its period and its sensitivity to light stimuli. According to the non-parametric model, the circadian rhythm entrains to light/dark cycles consisting of repetitive short light stimuli. When a stimulus falls at an appropriate phase of the rhythm, it can evoke a phase shift response that equals the difference between the circadian and the environmental period. The model was a big success with *Drosophila* mainly because the period and light-sensitivity were remarkably precise. However, it is important to note here that the period and light sensitivity in the *Drosophila* circadian rhythms are statistical averages of the period and light sensitivities of the constituent clocks of the individual flies, which explains why the period and light-sensitivity were so precise. Pittendrigh realized that nocturnal rodents were perhaps the most appropriate experimental animals to test the predictions from his nonparametric model based on *Drosophila* eclosion rhythm, because their circadian entrainment under natural conditions depends upon the interaction of two major aspects of the light/dark cycle (i) “dawn” when they retreat to dark nests, and (ii) “dusk” when they start foraging. The main challenge before him was to precisely measure the period and light-sensitivity in individual rodents, which lead to the first ever characterization of inter- and intra-individual variation in these two parameters.

In 1976 Pittendrigh published his work on mammalian circadian rhythms in five back-to-back papers in the *Journal of Comparative Physiology* A (Volume 106) which subsequently became citation classics. These five papers are a goldmine of information on the comparative circadian physiology of nocturnal rodents. They raise questions, propose models, and postulate hypotheses which will keep us engaged and enquiring for years to come. The studies described in these papers were primarily designed to provide a conceptual framework for the homeostasis of circadian period, to test the nonparametric model of entrainment and to analyze its implications in photoperiodic time measurement. Subsequently, in a series of behavioural studies on the circadian pacemakers underlying activity rhythms in four species of rodents, Pittendrigh demonstrated that the circadian pacemakers controlling activity rhythms in these animals were twice as precise as the overt activity rhythms. By carefully analyzing the activity behaviour of a large number of animals he described for the first time "memory effect" of prior environmental experience, which is also known as "after-effects" in circadian literature.

Pittendrigh encountered certain empirical irregularities concerning the interdependence of the pacemaker's circadian period, lability, and light sensitivity while making inter-species comparisons. This prompted the discovery of inter- and intra-individual differences in circadian parameters within species. It was this series of experiments that lead him to formulate the possible adaptive strategies available to natural selection for evolving biological clocks : not merely to measure the lapse of time (as in sun compass orientation), but more generally to recognize local time. The former function would require homeostasis of circadian clocks against temperature, nutrition and light, while the latter needs maintenance of a stable and reproducible phase-relationship between circadian pacemakers and external light/dark cycles. The challenges enforced by the inherent instability of pacemaker frequency are not so severe, because the pacemakers, as he had shown earlier with eclosion rhythm in *Drosophila*, were stable both in the face of daily environmental fluctuations and more importantly, against regular seasonal change of the entraining agent. Pittendrigh's studies in mammals demonstrated that the clock period was homeostatically buffered from alterations in light intensity as well, which motivated him to propose that the light-sensitivity and the lability of homeostatic conservation of clock period are functionally interrelated. Using the available data on clock period and clock's sensitivity to short light pulses Pittendrigh predicted the phase-relationship between circadian rhythms and light/dark cycles. He subsequently tested these predictions in a series of experiments in nocturnal rodents where he used different environmental manipulations to characterize entrainment. He discovered that the experimental data did not match the predications on several counts, unless he took into account inter- and intra-species variations in period and light-sensitivity. These series of experiments led Pittendrigh

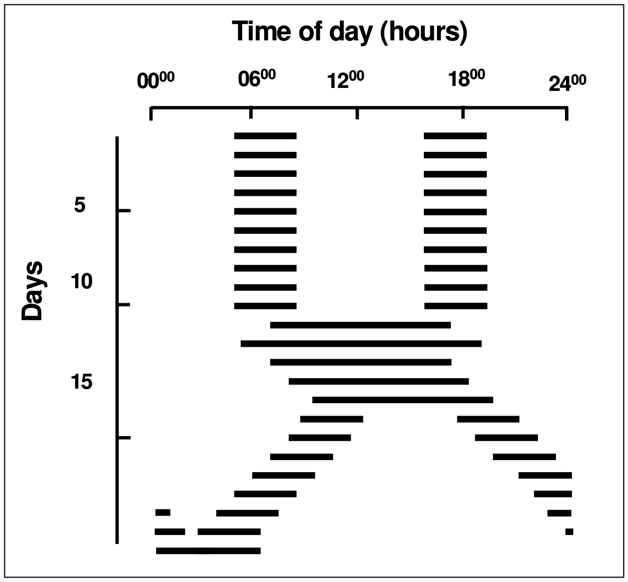

Figure 2. Splitting of activity rhythm. Activity/rest cycles of an animal in presence (first ten days) and in absence (last thirteen days) of light/dark cycles. The activity/rest cycles of the animal was bimodal, with high levels of activity around dawn and dusk and reduced activity at noon (siesta) and midnight (sleep). The activity/rest rhythms splits when the animal is transferred to continuous light, with the morning and evening bouts of activity free-running with different periodicities. Rest as in figure 1.

to put forward concepts such as "limits of entrainment", "minimum tolerable night" and "splitting". Many day- and night-active species show a bimodal pattern of activity with high levels of activity around dawn and dusk and reduced activity at noon (siesta) and midnight (sleep) (*Figure* 2). Such activity patterns are typically seen in organisms living under arid environments, temperate climates and sub-arctic regions. Furthermore, bimodality often continues after the animals are released into constant conditions without any external time cues. This suggests that the bimodal activity pattern is an inherent characteristic of the underlying internal clock(s) and not just dependent on environmental factors. Motivated by a phenomenon observed in the behaviour of some rodents where the activity rhythm of an animal splits into two or more components, thus simultaneously exhibiting two very different periodicities (*Figure* 3), Pittendrigh proposed the "morning-evening oscillator" model for circadian clocks. The model assumes that circadian clocks consist of two groups of oscillators with different responsiveness to light, one governing the morning and the other the evening activity.

I continue to read Pittendrigh's papers and every time find new questions and insights. The collection of the five papers which Pittendrigh published in 1976 is popularly referred as the

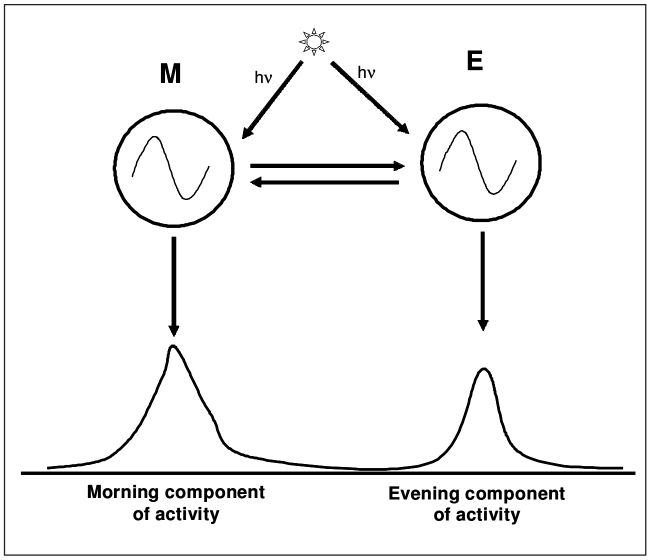

Figure 3. The Morning (M) and Evening (E) oscillator model of circadian clocks proposed by Colin S Pittendrigh. The model assumes that circadian clocks consist of two separate oscillators, the morning (M) and the evening (E) oscillators, with different responsiveness to light, one governing the morning and the other the evening activity.

"bible of chronobiology" and may be rightly regarded as guidelines for rhythm research of the future. Pittendrigh was a versatile scientist and an eclectic personality who nurtured the birth and development of a discipline for almost 45 years. His work lead to the development of clock models in insects and subsequently its extension to mammals. He was instrumental in providing the framework and guiding principles for the study of a unified model for circadian and photoperiodic clocks. As a teacher, researcher, philosopher of science, he enthused a large number of young minds who brought in the recognition and respectability which the field rightly deserved.

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Suggested Reading

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Glossary

Some technical terms, commonly used by chronobiologists, recur in some of the articles on Colin Pittendrigh. This glossary explains the terms.

D: Light/dark cycle.

LL: Continuous light.

DD: Continuous darkness.

Free-running period: Period of circadian rhythm in LL or DD.

Circadian Rhythm: Biological rhythm having a period of approximately 24hrs. Hence circa = about: dies = day. Circadian rhythms express innate period only during free-runs.

Zeitgeber (s) : Entraining or synchronizing environmental stimuli.

T: Period of the zeitgeber. 24 hours in the case of natural LD.

Entrainment : When a zeitgeber modifies a period such that it equals T.

Phase: Any point along the circadian rhythm.

Phase angle difference : The difference between a fixed phase of the circadian rhythm and a fixed phase of the zeitgeber.

Phase shift: Displacement of the free-running rhythm on the 'X' axis in response to stimuli such as light, temperature or chemical pulses. Phase shifts can be either advances or delays.

PRC: Phase Response Curve. A plot of responses of a circadian rhythm as phase shifts to perturbations as a function of phase.