Personal Reflections

Colin Stephenson Pittendrigh: A Memoir

Colin Pittendrigh was a dominant figure in the field of circadian rhythms, both by his strong personality and his extensive experimental contributions and interpretations thereof. His lectures were engaging, persuasive and typically much longer than scheduled. His research papers were similarly engaging and the prose elegant with ingeniously designed experiments and striking results. Many of his essays, such as "Perspectives in the study of biological clocks", as well as experimental studies, such as his landmark paper "On temperature independence in the clock system controlling emergence time in *Drosophila*" stand as classics.

Early Years

I first came to know Colin at Princeton University, where I entered as a graduate student in 1948. He had joined the faculty a year earlier, after completing his PhD at Columbia with Th. Dobzhansky as mentor. I was assigned as the teaching fellow for his undergraduate course in Invertebrate Zoology; the experience very much cemented our relationship. His lectures were elegant and erudite, demonstrating his extraordinary knowledge of the structure and evolution of species in the many phyla, knowledge that served him well many years later when he became the director of Stanford's Hopkins Marine Station in Pacific Grove, CA.

During those years the Pittendrighs lived at the Gray farmhouse on the outskirts of the town, not far from the lab. Colin and his wife Mikey welcomed graduate students at all hours; their home was a place for frequent informal gatherings with lively discussion on a wide range of topics, including how to teach evolution in the Sunday school that their children Robin and Sandy attended. They called it the "Atheist Sunday School".

Pittendrigh had spent much of World War II in Trinidad – as the song says, drinking rum and Coca-Cola – studying mosquitoes that breed in water pooled in epiphytic bromeliads in the forest canopy. This was part of a Rockefeller Foundation sponsored project aimed at the control of malaria. He observed daily rhythms in mosquito activity patterns, especially noting that peak activity times were different for different species, and at different canopy levels). This was certainly responsible in large part for the development of his interest in rhythms, which led to his experimental studies on the eclosion rhythm in *Drosophila*. Although he had not yet started benchwork on this by the time I left Princeton in 1951, the problem was very much on his mind. I remember very well discussions about biological oscillations with him in 1950 or before, seeking examples of enzyme systems, such as those on which I was then working, that exhibited oscillations in activity.

But throughout his life, the pervasive and persuasive theme of Pittendrigh's science was his perspective on evolution, profound and in some ways unique, and surely stimulated by his interaction with Dobzhansky. Prominent at the very outset of his clock studies was his interpretation of the phenomenon of "temperature-independence" in daily rhythmicity, now referred to as temperature compensation. Previous studies by Bünning showing a relatively small effect of temperature on the period of rhythms had been used, quite reasonably, to assert that temperature did have an effect on the period, and thus that the timing of rhythms was due to a biological phenomenon, not an exogenous factor, as was later also promoted by Brown. While a meaning for the relatively small effect of temperature was not perceived by earlier workers, Pittendrigh proposed it to be of functional importance. In doing so, he introduced the concept of an evolutionarily selected "biological clock", thereby captivating the interest and imagination of biologists. Pittendrigh argued *that a* clock whose timing was affected by temperature would be of little or no value to the organism. He inferred the existence of a functional biological clock, and was thus the first to put "... the problem of temperature-independence "... in ...its proper perspective. Albert Szent-Ġyorgyi is credited with saying "Research is seeing what others have seen but thinking what no one else has thought."

I Move to Johns Hopkins University

In 1951 I moved to Johns Hopkins University as a postodoctoral fellow in the laboratory of Bill McElroy, where I worked on the firefly luciferase system. During those years I sometimes stopped in Princeton for an evening at the Gray farm, for discussions of research and his by-then active work on *Drosophila*. In 1953 my wife to be, Hanna, accompanied me, and for our wedding Colin and Mikey gave us a copy of Erich Kahler's book, "Man the Measure". At the time he had written the book in 1943, Kahler's view of evolution included the notion that it should result in an improvement, which in the case of man's behaviour he questioned. A decade later he encountered Colin and the Sunday school lessons.

At Northwestern University

In 1953 I took a position on the Biological Sciences faculty at Northwestern, where Frank Brown was chairman. Brown was a highly accomplished, personable and well respected invertebrate endocrinologist who had been studying the remarkable daily rhythm of pigmentation change in the fiddler crab, *Uca*. Brown was to become a tenacious and ultimately outrageous advocate of a theory of exogenous control of the timing of rhythms, a theory that Bünning had challenged decades earlier (when mitogenetic radiation was claimed to be responsible). Brown considered a number of different geophysical variables as potential zeitgebers, as we now call them, but never fixed on one. One reason for his adherence to such a theory was the lack of effect of temperature on the period of the rhythm. He had reported that *Uca* exhibited a rhythm with a Q10 of exactly 1.0, but this was based on data taken by visual estimation (not double-blind) of pigmentation on a 5-point scale, and thus not very precise. Pittendrigh's 1954 determination of a Q10 of about 1.03 for the *Drosophila* eclosion rhythm illustrated how close to 1.0 a value might be, and made it clear that the value for *Uca* could also be slightly different from 1.0.

Hans Kalmus, reported in a short letter to *Nature* effects of light and temperature on the rhythm of eclosion in *Drosophila*. Kalmus, a refugee from Nazi Germany, gave clear descriptions of experiments in which he found that the rhythm was "inherent", synchronized by light and persisting in constant dark with periods not greatly different at different but constant temperatures. Pittendrigh criticized Kalmus for failing to see the meaning of the results, which was understandably difficult because of Kalmus' (also correct) observation that the first period after a temperature step is highly temperature-dependent, interpreted by Pitt as a transient effect. Kalmus had also reported that a single brief light exposure would initiate a rhythm in arhythmic flies. Although he did not continue to work in the field after 1940, he came to a Gordon conference on Chronobiology in the 1980s, stayed overnight at our home in Cambridge and talked the ear off Colin, who also stayed with us.

At Northwestern I had no idea that I would enter the circadian field. And it was neither Pittendrigh nor Brown that brought me into the field, but the bioluminescence of dinoflagellates. At Northwestern I was working on bacterial luminescence and delivered a paper at a meeting on bioluminescence at Asilomar in Pacific Grove, CA in April 1954. Francis Haxo, who I knew well from Hopkins, where he had been on the faculty, but was then at the Scripps Institution of Oceanography in La Jolla, delivered a paper with Beatrice Sweeney, alias Beazy, on the luminescence of her newly isolated culture of the marine dinoflagellate *Gonyaulax polyedra*, with evidence that it was rhythmic.

Anxious to study the biochemistry of a new and different luminescent system, I arranged with Francis and Beazy to spend the summer of 1955 in their lab for that purpose. The work was successful and richly rewarding, but we could not restrain ourselves from also studying the properties of the daily rhythm, leading to the first determination of a phase response curve (PRC) for shifting the phase of the rhythm and the action spectrum for phase shifting, showing peaks in both the blue and red. That work and later studies established that the luciferase enzyme was present in extracts made during the night but absent in daytime extracts, correlating with the in vivo rhythm. It would be many years before we could demonstrate that this was due to a daily synthesis and destruction of the enzyme.

Enter *Gonyaulax*

Continuing at Scripps the next year, this time on a sabbatical for 6 months, we were greatly surprised and delighted when Colin showed up. He was being courted by Scripps for an appointment as professor there and had been enticed to spend two weeks in residence. He came to be very interested in the position, but Princeton recognized that he should be treated better, and they did so. For us it was glorious to spend time with him discussing science and clocks and our new *Gonyaulax* system.

In 1956 we were anxious to see how the *Gonyaulax* rhythm behaved at different temperatures, so we took our cultures to Cal Tech and used seven temperature controlled rooms in Fritz Went's Phytotron, measuring the flashing rhythm for eight days. A major finding was that the period was measurably different at different temperatures, much as had been reported by Bünning in *Phaseolus*. The effect was also not great, only about 15% different over the range of 16° C to 26°C. But unlike *Phaseolus*, the clock ran slower, not faster, at higher temperatures, suggesting over compensation, which we modeled by two opposing temperature dependent reactions. If a temperature compensation mechanism is involved and it is not exactly compensated, over compensation is as likely as under compensation. This mechanism is now widely accepted, with the two reactions corresponding to the positive and negative elements in a feedback loop. This finding also led to the abandonment of the term "temperature independent" in reference to circadian rhythms in favor of "temperature compensated".

Pittendrigh was largely responsible for planning and organizing the memorable Cold Spring Harbor meeting in June of 1960, where he and Brown openly locked horns. Four years later Jürgen Aschoff convened a two week-long NATO summer school on Circadian Clocks with a large attendance and many students. Brown again elaborated on how the external geophysical signals could provide the timing information for the clock. He had dubbed the mechanism "autophasing", which came to be dubbed "phase (pronounced face)-saving", which he did not appreciate. Pittendrigh ignored the matter in his formal presentation on entrainment; the controversy no longer commanded major attention.

Pitt and Mikey Move to Stanford

A year later, in 1965-66, I took a sabbatical leave, working at Rockefeller University, but living in Princeton. Colin had been appointed Dean of the Graduate School that year but continued with active research and teaching. Despite his increased load, I got to see him from time to time and on some occasions had extended conversations with him alone. With his basic love for and commitment to scholarship and teaching, he was conflicted as an administrator. In my view he had very good ideas and made important contributions to that task, but when a few years later Don Kennedy proposed that he take a professorship at Stanford, with new challenges for teaching and good opportunities for research, it was a way for him to gracefully withdraw. He and Mikey moved and never regretted it.

The Pittendrighs at Pacific Grove

In 1976 Colin accepted the Directorship of Stanford's Hopkins Marine Station in Pacific Grove. With only a few other professors in residence at the station administrative duties were quite modest, and he was able to devote almost full time to his research, and to work with a number of brilliant collaborators over the years. In 1977 he organized and hosted a summer workshop on Circadian Clocks at Hopkins in which students and scientists from all over the world participated over the course of 6 weeks. I still have a six-inch-thick folder of papers and notes from that meeting collected by one of my students, three of whom attended, along with several former students.

On his sixty-fifth birthday his friends organized a surprise party at the station in which his friend Jürgen Aschoff and company came ashore on the beach at the back of the lab in a boat flying the ensign of a pirate. Guests sprang forth from hiding, and Mikey joined in the fun, which continued at his home late into the night. Colin was a great scientist, an inspiring teacher, a wonderful colleague and a lifelong friend. His contributions and his camaraderie will long be remembered.

Suggested Reading

[1] C S Pittendrigh, Perspectives in the study of biological clocks. In *Perspectives in Marine Biology: A Symposium*, A A Buzzati-Traverso ed., University of California Press, Berkeley, CA, pp. 239-268, 1958.

[2] H Kalmus, Diurnal rhythms in the axolotl larva and in *Drosophila*, *Nature*, Vol.145, pp.72-73, 1940.

[3] B M Sweeney, and J W Hastings, Characteristics of the diurnal rhythm of luminescence in *Gonyaulax polyedra*, *J. Cell. and Comp. Physiol.*, Vol.49, pp.115-128, 1957.

[4] E Bünning, Physiological mechanism and biological importance of the endogenous diurnal periodicity in plants and animals, In *Photoperiodism in Plants and Animals*, R B Withrow, ed., A.A.A.S. Press, Washington DC, pp. 507-530, 1959.

[5] J W Hastings, and B M Sweeney, On the mechanism of temperature independence in a biological clock. *Proc. Natl. Acad. Sci.*, USA, Vol .43, pp.804-811, 1957.

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