Unfortunately, I am not qualified to render an eye-witness history of Colin Pittendrigh's remarkable career; indeed, many of his seminal achievements – the discovery of the circadian clock in *Drosophila*, the characterization of the common characteristics of circadian clocks, the debates with Frank Brown over whether circadian timekeeping is intrinsic or environmentally driven, the first formal models of circadian rhythmicity – were all history at my birth. All I can offer is a small vignette of Pitt in his later years, of the 'lion in winter'. By a process of pure serendipity I had the pleasure of becoming perhaps Pitt's last (albeit informal) student. Our educational model was ahead of its time – a combination of e-mail correspondence and one remarkably intensive weekend seminar. The dialogue we held was representative of the great currents running through the field in the late 1980's and early 1990's, currents which are still manifest 15 years later.

My first and only interaction with Pitt in a classroom was a guest lecture he gave to my undergraduate organismal physiology course during my sophomore year at Stanford in 1983. He lectured on avian migration mechanisms, how some birds measure the position of the sun against their internal clock to determine the direction of migration. Pitt was a powerful lecturer; this was one of the few lectures I remember well from this course (now 23 years ago!). (Interestingly, Pitt later claimed that hearing Gustav Kramer lecture on the same topic in 1951 stimulated his own interest in clocks). Pitt retired the next year, and – were it not for serendipity and the kindness of a wonderful lab technician – I probably would have had no interaction with Pitt again.

I had stayed at Stanford for my medical and graduate school training, and was working in the labs of William Dement and...
Jack Barchas. I had become very interested in circadian clocks. Circadian rhythms are the nearly 24-hour rhythms of physiology and behavior that are an almost ubiquitous feature of eukaryotic life. When I began my graduate career, very little was known about the molecular mechanisms underlying circadian clocks. At that time – 1988 – I had initiated a project asking the question: What genes are expressed with a circadian rhythm? A growing body of evidence suggested that timed gene expression might be important in the mechanism of the circadian clock. For example, the application of inhibitors of protein or RNA synthesis to the fungus Neurospora would reset the circadian clock, but the resetting effect depended on the time of day that the drugs were applied. Jennifer Loros and Jay Dunlap at Dartmouth had recently discovered several genes expressed with a circadian rhythm in Neurospora (this work’s lineage could be traced back to Pittendrigh’s student Jerry Feldman). I had started this project in the mouse, but when one of my advisors moved universities, I was fortunate to be able to transfer the project to Drosophila in Mark Krasnow’s laboratory.

Enter serendipity. Evelyn Parker was Pitt’s last technician at Hopkins Marine Station, Stanford’s marine laboratory in Monterey, California. Pitt set up his lab in that beautiful location several years after he moved from Princeton to Stanford in 1970. When Pitt retired, Evelyn found employ in Mark Krasnow’s lab, and so we ended up bench neighbors during my first year in the lab. When Evelyn learned of my project she relayed this to Pitt (with whom she had stayed in touch). On Pitt’s next visit to the Bay Area (by this time he was splitting his retirement time between Sonoita, Arizona, and Bozeman, Montana) we found time to get together.

I knew that Pitt had founded the field of Drosophila circadian rhythmicity; one could claim he founded the whole field of biological timekeeping, although I am sure Pitt would have argued that he followed in the footsteps of Bünning and Kalmus. Pitt came to circadian physiology through botany, becoming interested in biological timing in the course of war-time studies of mosquitoes living in bromeliad leaves; different anopheline mosquito species became active in the trees at different times of day. After World War II, Pitt trained in Theodosius Dobzhansky’s laboratory at Columbia, and his thesis work focused on the daily rhythms of activity of two Drosophila strains, D. pseudoobscura and D. persimilis in the forests of the Northern Californian Sierras. Dobzhansky was one of the great evolutionary geneticists of the 20th century, a fierce defender of evolution who was responsible for the dictum, “nothing in biology makes sense except in the light of evolution”. This evolutionary perspective was deeply instilled in Pitt, and remained an essential part of Pitt’s science throughout his career.

In the early 1950’s, Pitt discovered that the eclosion of Drosophila pseudoobscura from pupa to adult is regulated by a free-running circadian clock (Figure 1). He quickly demonstrated that
the period of this rhythm was relatively temperature independent (an experiment famously carried out in the Rocky Mountains using an abandoned outhouse as a darkroom and a pressure cooker submerged in a nearby stream as a cooler).

The finding – that the clock was temperature-compensated – was among the first pieces of evidence for a defining principle that Pitt promulgated throughout his career: the mechanism of the circadian clock is separate from those of the behaviors it controlled. This seminal insight – that the timing of eclosion, for example, was largely independent of the details of the eclosion process – implied that the properties of the clock (the driving oscillation in his terms) could be studied independent of whatever physiological function it was controlling. The same insights to clock mechanism could be gleaned from studying hatching as locomotor activity. The underlying clock thus has its own, conserved properties. Pitt devoted a great deal of his lab-life to understanding these clock properties, initially taking a broad comparative
physiology approach. Pitt first performed a large series of experiments to demonstrate that circadian rhythms are intrinsic to the organism and independent of external cues. He carried on a famous and protracted debate with Frank Brown, Jr. of Northwestern University, who felt that observed circadian rhythms were driven by environmental periodicities. Pitt’s data and arguments ultimately prevailed.

Pitt studied circadian clocks in across a menagerie of organisms from unicellular *Euglena* to arctic voles. With his students, Pitt (and independently Jürgen Aschoff in Germany) generated a rich language for circadian rhythms based on oscillator theory. Terms used widely by circadian biologists, including rhythm amplitude, period (tau), phase resetting paths, limit cycles, oscillator coupling, master and slave oscillators, all originated in the work Pitt initiated in the 1950’s. In 1960, Pitt distilled a series of empirical generalizations about circadian rhythms that hold true across all organisms. With one exception, these tenets hold true 45 years later, and largely defined the field of inquiry for a generation.

The field began to slowly shift in the late 1960’s. In 1968 Ron Konopka, a graduate student in Seymore Benzer’s lab at Caltech, initiated a mutagenesis screen in *Drosophila melanogaster* looking for single genes that influenced circadian rhythms in the fly. This experiment made the implicit assumption that the circadian clock had a discrete genetic (and by extension, biochemical) basis; it also relied on Pitt’s hypothesis that one could alter the circadian clock without altering the functions, such as eclosion, it controlled. The notion that a single gene could specifically control a physiological system as complex and widespread as the circadian clock was nearly heretical. Remarkably, Konopka isolated three alleles of a single gene – named *period* or *per* – that respectively slowed, sped, or stopped the circadian clock altogether. (Indeed, this notion was so heretical that when Konopka and Benzer told Nobel Laureate Max Delbrück of these results, he said, “It is impossible...I don’t believe a word of it”). These experiments, as well as experiments that followed shortly after by Pitt and Victor Bruce’s former student Jerry Feldman showing comparable genes in *Neurospora crassa*, gave the first indication that circadian rhythms have a discrete genetic basis.

Pitt was intrigued by the *per* mutants; Konopka spent time with Pitt analyzing the formal aspects of their clocks. While the mutants were helpful in confirming some of the formal properties of the clock – fulfilling a prediction as to the relationship between the amplitude of a circadian rhythm and its temperature compensation, for instance – little insight came from these flies as to the molecular mechanisms of circadian timekeeping. These mutants were ‘ahead of their time’ (pun intended) as the molecular techniques to understand genetic function were in their infancy in the 1970’s. In the early 1980’s, two groups took up the task of trying to provide molecular insight into the genetics of clock mutant flies. The technique
of 'chromosome walking' – injecting embryos with a series of overlapping plasmid or cosmid DNAs to create transgenic flies that might show rescued phenotype – had been developed in the early 1980's. Michael Rosbash and Jeff Hall at Brandeis University, and Michael Young at Rockefeller University decided to apply this to the per gene. Both groups cloned the same mutant gene. Unfortunately, however, the sequence of the per gene again gave essentially no insight into its function.

This epoch in the field saw a great divide among three groups: the molecular biologists working in this nascent domain, who had the belief that understanding of structure and function of genes critical to circadian rhythmicity would yield mechanistic insight; comparative empiricists who felt the clock mechanisms would be apparent with more and better physiologic experiments in diverse organisms; and the ‘formalists’ who felt that mechanistic insight would come from understanding the mathematically formal and model-able properties of the circadian oscillator. Pitt – although contributing mightily to the middle group – was an avowed advocate in the latter, writing in 1960, “To make progress analyzing circadian rhythms we must perceive what the problems are – or rather state what we take them to be – and proceed with accumulation of new information only as it tests, and alas, probably eliminates, theory.”

Antagonistic relationships in science usually have only two sources. First, individuals can have axiomatic differences – that is, they believe in different tenets which are equally unprovable (but can have very different ramifications!). Second, antagonism arises when individuals cannot communicate because of language or cultural barriers. Both were in place here. Each camp axiomatically believed that their approach would ultimately yield the most useful understanding of the clock; each also believed their language was uniquely suited to describing the clock. Early on, many in the molecular biology camp had difficulty understanding the idiosyncratic jargon of circadian oscillator theory; and the mysteries of genomic cosmid libraries and P-element transformations were equally opaque to those raised on circadian physiology. At this time, Pitt was squarely in the camp of the ‘formalists’; and warm feelings between Pitt and at least some of the molecular biologists were lacking.

At about the time I moved to Mark Krasnow’s lab, and a little before I began my tutorial with Pitt, the ‘Rosetta Stone’ experiment of circadian physiology – connecting molecular genetics with formal clock theory – was published by Paul Hardin, Jeff Hall, and Michael Rosbash at Brandeis. Paul discovered that the period mRNA oscillates with a circadian rhythm in the fly head, with mRNA levels low during the day and peaking in the early night. What Paul and colleagues noted was that the transcript of the arrhythmic allele of period, per\textsuperscript{lo}, did not oscillate in a per\textsuperscript{lo} fly. Yet when Paul introduced a wild-type copy of the period gene into the per\textsuperscript{lo} fly, this
rescued rhythmicity in both the endogenous mutant and wild-type (introduced) per gene transcripts. The group thus demonstrated that the period gene regulates its own circadian rhythm of expression; this constitutes a formal feedback loop which could be the mechanistic basis of a clock.

In subsequent years, this fundamental model has been significantly expanded. In the fly, at least six genes directly and specifically affect the circadian clock: period, timeless, clock, cycle, vrille, and CKIε. These genes participate in a transcription-translation time-delayed feedback process, where the Period and Timeless proteins essentially repress their own expression by blocking the ability of Clock and Cycle to promote transcription from the period and timeless gene promoters. A similar mechanism was discovered for Neurospora by Jay Dunlap, Jennifer Loros, and colleagues. This ‘TTL’ (transcription-translation loop) oscillator is now the standard model for clock function in eukaryotes. In the late 1990’s, homologues for nearly all the fly clock genes were found in mammals. Reverse and forward genetic experiments in mammals showed that these genes function quite analogously in the mouse as they do in the fly (with substantial differences in the details, however).

At our first meeting in 1990, Pitt came to Mark Krasnow’s lab in the CMGM building at Stanford Medical School, and we had lunch outside on the patio, absorbed in discussion of Hardin’s experiment and implications of circadian gene expression studies. Pitt was particularly interested in understanding the mechanisms of transcriptional regulation in the fly; he had never done molecular biology research, and was unfamiliar with precise meanings of terms like promoter and enhancer. So here I was, a second year graduate student, giving a tutorial on molecular biology to the founder of my field and a member of the National Academy of Sciences! Soon, however, the tables turned and Pitt again became the teacher, discussing many of his seminal experiments in rapid fashion, his northern-English brogue becoming quite animated. I struggled to keep up. We parted promising to stay in touch. Over the next year, we exchanged frequent correspondence in which I sent Pitt results of my experiments, and he taught me a great deal of Drosophila circadiana.

However, I was truly surprised when, in 1991, he invited me to visit with him at his winter home in Sonoita, Arizona. We had both been invited to attend the inaugural meeting of the Center for Biological Timing, at the University of Virginia. Could I come down to Arizona first, and we could travel together to Virginia? Of course I leapt at the opportunity.

Pitt met me at the airport; we went out to lunch in Tucson on Reuben sandwiches. We then drove the 30 or so miles to his home in Sonoita. It was a small house on a bluff facing south. From his front yard, one could see four or five mountain ranges fading into blue outlines in
Mexico. No other house was visible in any direction. Pitt's wife Mikey was absolutely gracious in welcoming the young stranger into her house.

The next two days were spent in the most intensive tutorial I have experienced. Pitt and I tried to reconcile the feedback oscillator implied by Hardin's results with the formal phase resetting properties of the clock. I would suggest a property predicted by genetic feedback, and Pitt would say to me, "But chum, you can't have strong resetting to weak stimuli in that model" or similar retort; back and forth we went. I wish I could say that we emerged with a coherent molecular mechanism explaining the observable properties of the circadian clock, but this remains a holy grail 15 years later (although much progress has been made). However, two important things did happen during that weekend. First, Pitt came to embrace the notion that a molecular genetic mechanism underlies the circadian clock, a notion that is now firmly established. Second, I received the education of a lifetime. Most tempting were Pitt's file cabinets in his study. At various times during our discussions, he would say, "We did that experiment in 1963 or 1964 but never published it - let me find the data", and he would dig through a drawer and find a hidden gem - "Here it is - see, Drosophila pupae grown under pure nitrogen will phase advance but not phase delay, so there must be different metabolic mechanisms of phase advances and phase delays". I wondered how many other truffles were hidden in those drawers. I suspect Pitt would have let me have free run over his unpublished data had I asked; I have had the occasional pang of regret that I never did so.

We traveled together to the meeting in Virginia, me happily serving as his porter as advanced asthma had limited his mobility. I saw Pitt a few more times after that; at the 1993 Gordon Conference, where he gave his final, powerful lecture on 'Temporal Organization: Reflections of a Darwinian Clock-Watcher'; we met once in Monterey, his old stomping grounds, and once he stayed with my then-fiancée Suzy and me in our small Menlo Park apartment. When Suzy and I married in 1994, Pitt sent a small silver box as a gift, which we proudly display today in our home. My last contact with Pitt was in late 1995, when he called to ask my opinion on a paper that he was editing for the Proceedings of the National Academy of Sciences. Pancreatic cancer took his life in early 1996.

Pitt was a remarkable scientist and a remarkable man. Having now worked in a number of fields outside circadian rhythms, I have yet to come across any other scientist who so dominated the intellectual content of his or her field for so long. My colleagues and I continue to read Pitt's 50-year old papers and find fresh insight in them. They are remarkably tightly written; clearly the author realized these would be touchstones of the field far into the future. Pitt also had an enormous personal influence on me - yet our sum 'face time' was only a few days! Although Pitt's very strong personality polarized a number of his relationships, his
legacy in outstanding students who have led and continue to lead the field, including Michael Menaker, Arthur Winfree, Jerry Feldman, Serge Daan, Carl Johnson, and many others, has been enormous. Pitt's impact in understanding biological timekeeping will be manifest as long as there is an active field of inquiry in circadian rhythms.

Suggested Reading

[6] http://stke.sciencemag.org/cgi/content/abstractsigtrans;2003/194/cm10

J C Bose and Diurnal Rhythms in Plants

Not very widely known to chronobiologists are the first class contributions of Jagadish Chandra Bose (1858-1937) on diurnal rhythms in plants. Bose was a professor of physics in Presidency College Calcutta and had himself summarized his extensive researches in physics and the topics of responses of living and non-living objects and plant physiological investigations in several learned monographs. In the monograph Life Movements in Plants (in three volumes written in 1918, 1919 and 1923) Bose described entrainment of plant leaf movements to light: dark cycles and observed free-running periods in continuous light and constant darkness (M K Chandrashekaran, J C Bose's contributions to chronobiology, Resonance, Vol. 3, No.2, pp.53-61, 1998).

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