Biological rhythms research: A personal account

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1. Introduction

It has been my good fortune, owing to post-doctoral research years in Bünning's and Engelmann's laboratories at the University of Tübingen, and at the University of California in Berkeley, to have enjoyed a close personal and professional relationship with many eminent rhythm researchers. When I once boasted about this, a younger and very competent colleague blurted out "God, you have known all the big boys in the game!". Hence this essay of rambling thoughts and anecdotes, the chief virtue of many of them being that they are not available in print. What follows is an unabashedly subjective account of developments in this field as perceived by me and has no pretensions to being a comprehensive history of biological rhythm research (for an early account see Bünning 1960).

Chronobiology is a relatively new name for a very old subject. It is the discipline which involves investigation of biological rhythms occurring in some prokaryotes and in all eukaryotes from fungi to humans. Rhythms in the physiological processes may have 24 h, 29 day and 365 day periods and are called *circadian*, *lunar* and *annual* rhythms respectively and represent adaptations to the geophysical periodicities which exist in nature. The most ubiquitous of these rhythms are the *circadian* rhythms which are also called biological clocks. Unfortunately the word 'chronobiology', even though convenient (in as much as it covers the several biological rhythms), has been identified as representing a particular school of thought of which Franz Halberg is seen as the leader. Halberg developed a whole complicated glossary related to chronobiology and statistical methods for its study involving specially coined terms (acrophase, sinor, etc.), none of which has found wide acceptance. Erwin Bünning's first book on biological rhythms (Bünning 1958) does not use the word chronobiology, nor do its subsequent English and German editions. A 1965 monograph on the subject by A Sollberger was entitled 'Biological Rhythm Research'. An important society in the USA, founded in the 1990s, is called 'Society for Research on Biological Rhythms'. However, there is no scientific orthodoxy militating against the use of 'chronobiology' to describe the subject of study of biological rhythms; in fact there is a speciality journal called *Chronobiology International*. Bünning wrote in 1977 in the preface to the third revised German edition of his book '*Die physiologische Uhr*' (free translation): "Until two decades ago one got to hear (or even to read) that the assertion of the existence of endogenous daily rhythms belonged to the realm of metaphysics. The proposition that this 'endogenous clock' was really employed by plants and animals to measure biological time was taken to indicate that biology had again lapsed into mysticism and parapsychology". Bünning was never given to exaggeration. His words point to the precarious conditions under which early rhythm researchers had to work. Why a perfectly defensible area of biological inquiry was treated as the soft underbelly of biology deserves to be made the subject of study by students of the sociology of science. Part of the reason may have to do with developments that often distracted and hindered progress as mentioned later in this essay.

2. History

The study of biological rhythms has a hoary scientific tradition. It drew into its fold many brilliant scientists in the 18th, 19th and 20th centuries. The first recorded circadian rhythm was for the sleep movements of the

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leaves of the tamarind tree by the Greek philosopher Androsthenes when he joined Alexander of Macedon in his march on India in the fourth century BC. Carl von Linné (1707–1778) constructed a 'floral clock' based on his knowledge of the opening of flowers at different hours of the day. The French astronomer de Mairan performed in 1729 a blemishless experiment in the modern scientific mode and established that the 'sleep' movements of the touch-me-not plant (*Mimosa pudica*) were endogenous: de Mairan removed these plants into the perpetual darkness of deep caves and demonstrated that the movements persisted.

As early as the second half of the 19th century, the tradition of research on biological rhythms, as indeed on all branches of biology, was strong in Germany and the rest of Europe but not as strong everywhere until much later. F A Brown Jr (1908–1983) in the USA has written that he had not divulged to anyone his scientific interest in biological rhythm research until he had secured a tenured job. This, for example, was not necessary in Germany, notwithstanding the prevalent scepticism of the scientific establishment. Wilhelm Pfeffer (1845–1920) had a laboratory built at the University of Leipzig nearly 100 years ago, which even by today's standards was modern and state-of-the-art. He had not only constructed a room with constant temperature himself, but also had rooms with automatic switching to provide alternating light and dark periods, including simulation of dawn and dusk conditions. J C Bose (1858–1937) wrote significant papers (in English) on his findings on diurnal movements of leaves of plants. He discovered the entrainment of the movements to light : darkness cycles and observed free-running periods in continuous light and continuous darkness as early as 1919 (Bose 1919). This work was cited by Bünning (1958) in his first monograph on the subject of biological rhythms. Early this century Semon (1905, 1908) argued in favour of the genetic inheritance of circadian rhythms. The Dutch botanist Antonia Kleinhoonte (1929), working with the leaf movements of the large jack bean *Canavalia ensiformis*, reported entrainment by light : dark cycles and free-running in continuous light. In 1930 Bünning and Stern stressed that the periods of rhythms under constant conditions deviated from 24 h thus justifying the use of the term circadian.

A word here about where German scientists generally published right until the Second World War. The botanists published in *Annalen der Botanik*, the zoologists in *Annalen der Zoologie*, the physicists in *Annalen der Physik* and so on. It is a distinctively post-Second-World-War phenomenon that where something is published is more important than what is published. But then in the USA, even literature published in English but in European journals, was not often known or cited. Pfeffer published his important papers of 1907, 1911, and 1915 on diurnal rhythms in plants in *Abhandlungen der mathematisch-physischen Klasse der Königlich-Sächsischen Gesellschaft der Wissenschaften*, a journal not readily available in university libraries. Bünning was to comment that the papers were given 'a first class state funeral' (*ein Staatsbegräbnis erster Klasse*).

The eloquent term *circadian* was coined in 1959 from the Latin *circa* and *dies* by Halberg (1959). Until then there was a welter of names for these daily rhythms. The English favoured diurnal, the Americans daily, the Germans (closer to the point) endodiurnal, and the Canadians diel. Rhythms were themselves called cycles, periods, and periodicities. The Cold Spring Harbor Symposium on Quantitative Biology of 1960 on 'Biological Clocks'—a bold title—brought together practically everyone who was (or was going to be) anyone in the field and signalled the beginning of the modern age in rhythm research. The galaxy of biological rhythm researchers that participated succeeded in putting an end to nomenclatural wrangling. Unfortunately Gustav Kramer, who had shown that birds consult in homing as well as in migration the position of the sun with the aid of their biological clocks, had died the year before. Pittendrigh (1993) has written that it was a lecture he heard being delivered by Kramer in 1952 that helped him to make the crucial transition of thinking in terms of *clocks* instead of *rhythms*. 'Clocks' would continue to be shunned well into the 1980s by a few for their mechanistic implications. This symposium also helped further to firm up the cordial relations that existed till the last between Aschoff, Bünning and Pittendrigh.

3. Some personalities

The history of any subject would be soulless if the names of its prime movers were not woven in. I give here thumb-nail portraits of a few of the most influential rhythm researchers I have personally known. A large number of those who participated in the 1960 Cold Spring Harbor Symposium deserve to be so honoured but it is hardly possible to undertake the task in a brief essay such as this. That said, the choice of personalities is arbitrary and most certainly influenced by my personal relationship with them and the great esteem in which I hold them.

3. *Jürgen Aschoff (1913–1998)*

Jürgen Aschoff was born on January 25, 1913, in Freiburg in Breisgau (Germany) as the fifth child of the pathologist Professor Ludwig Aschoff and his wife Clara. He obtained his MD in 1937 and Doktor Habilitation in 1944 and became a Professor of Physiology in 1949 at the University of Göttingen. He came into close contact with

Gustav Kramer, who a short while earlier had discovered the sun compass orientation in birds, and with Erich von Holst (1908–1962) from whom he acquired a knowledge of the coupling of oscillators and relative coordination. He has stated (Aschoff 1990) that it is due to the interest that these two great and charismatic scientists took in his work in 1958, that he could become a member of the Max-Planck-Institut für Verhaltensphysiologie which had just then acquired new buildings at Seewiesen in southern Bavaria. Aschoff changed over from medically oriented physiology to biology at large with its wider horizons.

Soon after Aschoff was appointed the Director of the sister Max-Planck-Institut für Verhaltensforschung (MPIV) at Andechs just a few miles away from that of Konrad Lorenz, Andechs became the Mecca for chronobiologists. Aschoff drew his largest contingent from the USA. He had built the first 'isolation facility for the study of human circadian rhythms' at the MPIV early in the 1960s. I first set eyes on this bunker in the company of Pittendrigh, W Engelmann and H-W Honegger, little realising that I would be building one myself at Madurai Kamaraj University many years later.

Aschoff's contributions have mainly been in the area of unravelling the physiological mechanisms that regulate circadian rhythms in birds, mammals and humans. His publications provided the tools for the rigorous analyses of biological rhythms, adapting terminology and analogies from oscillation theory dealing with physical, chemical and mathematical oscillators. He also precisely defined the behaviour of circadian rhythm parameters in light-entrained and free-running states in what have been called Aschoff's Rule and the Circadian Rule (Aschoff 1960).

J Aschoff and C S Pittendrigh

There was a time when his rich-timbred, bold voice would scare timid young scientists. Aschoff reassured me in 1979 during a meeting of the International Society for Chronobiology in Hanover that "... just because I talk in a loud voice people mistake me for an arrogant man, which I am not". The pipe-waving and argumentative Aschoff of the first two decades of the MPIV did come across as someone who wanted to pull his opponents down. He appeared to be self-assured to the point of being cock-sure on clock matters. He had also attracted to his centre other brilliant researchers who, as a team, came into a small seminar hall one by one, sat helter-skelter and seemed to go after the hide of their seminar speakers. I remember this hall as being a cold, unheated room. I had never picked up the courage to speak in it myself but do recollect a seminar Engelmann and Honegger gave on the Ph.D. thesis contents of the latter. Each statement that was made was dissected, every slide discussed and every conclusion questioned. Honegger was one of Bünning's first students but Engelmann had virtually supervised Honegger's work and therefore felt personally responsible for every perceived weakness in the thesis-in-the-making. The topic was the *Drosophila* eclosion clock, on which I was working myself with generous help from Engelmann. Honegger had proposed an "on" and "off" model to explain the time course of the rhythm. The model was not entirely satisfying for it could only "explain" acquired data and was wanting in the realm of being able to make predictions. I used to tease Honegger that his model was "*mal-an und mal-aus*" (sometimes on, sometimes off). I consoled him saying that just because R Wever and K Hoffmann were aggressive did not mean that they really understood and the piece of work was, after all a Ph.D thesis. Klaus Hoffmann was a Prussian, brilliant, articulate and very sarcastic. He had demonstrated, working in the Max-Planck-Institute of G Kramer in Wilhelmshaven, that migratory birds did use their biological clocks for orientation in time and space. If he trapped the birds and experimentally shifted their light : dark cycles by 6 h, the birds when released made a 90° error in the direction they took off.

For all his air of self-assurance, now and then Aschoff was given to soul searching, diffidence and doubts. In April 1967 in Göttingen he wondered in the company of Engelmann, Honnegger and me if he were not after all "playing the glass bead game" referring obviously to the various tenets and models of his such as the Circadian Rule, Aschoff's Rule and the Aschoff–Wever Model. Aschoff was quick to see virtue in the work of other scientists, even in much younger scientists. He carefully read the papers of others, in contrast to many scientists in the USA, Europe and elsewhere. For thirty years after wondering if he was playing the glass bead game, having provoked and deeply influenced many younger chronobiologists into making newer and newer discoveries, Aschoff was active and remained so right until his death on 11 October 1998 after a brief illness.

3.2 *Erwin Bünning (1906–1990)*

Within a few weeks of the passing away of Erwin Bünning on 4 October 1990 the German daily *Scwäbisches Tagblatt* described him as one of the greatest botanists of the century. Pittendrigh drew the attention of English-speaking chronobiologists to the scientific contributions of Bünning, especially his entirely original idea that endo-diurnal rhythms acted much like yardsticks in seasonal time measurement such as photoperiodic phenomena. Bünning had found an adaptive function for circadian rhythms which Charles Darwin himself had not. Pittendrigh christened it Bünning's hypothesis. This postulate of Bünning first appeared in print when he was 30 years old, in a paper that was highly cited, becoming the most cited paper ever published in that journal (Bünning 1936).

The life and work of Bünning were shaped to a large extent by the forces, traditions, ideas and academic milieu prevailing in the schools and universities of Germany beginning with the nineteenth century (Bünning 1977). He was born on 23 January 1906 in Hamburg to the school teacher Hinrich Bünning (1878–1945) and Hermine Bünning née Winkler (1878–1954). Hinrich Bünning was a social democrat and firmly opposed to the Nazis. He taught German, English, mathematics and biology and was the earliest academic influence in his son's life. Bünning attended school in Hamburg from 1912–1925 and during the last school years had read "books which normally will be found only in university libraries". He studied biology, chemistry, physics and philosophy in the universities of Berlin and Göttingen between 1925–1928 and obtained his Dr. Phil. degree in 1929. Berlin was a stimulating place for a student of biology and was the work place of the Nobel Laureates Otto Warburg, Otto Meyerhof and Hans Spemann. Here were also one of the rediscoverers of Mendel's work, Carl Correns, the plant anatomist Gottlieb Haberlandt and the zoologist Max Hartmann. To join Berlin University as a student of natural sciences meant joining its faculty of philosophy, for most German universities still had the four traditional faculties: theology, medicine, jurisprudence and philosophy. Bünning's contemporaries at Göttingen were the physicist James Frank (24), the chemist Adolph Windaus (23), the zoologist Alfred Kuhn (23), the physicist Werner Heisenberg (22), Pascual Jordan (24), Otto Hahn (22) and Max Delbrück (24). Indicated within parentheses are the ages of these young men at the time.

There was far too much of philosophy and speculation instead of experiments in German universities in earlier epochs. This was partly due to the movement called *romantische Naturphilosophie* which held sway over German biology for long. In France and Great Britain experimental work in the 19th century very nearly replaced pure speculation in astronomy, physics, and biology. In Germany the influence of certain philosophers remained strong. Only a proper world of ideas was considered to lead to progress in learning about the external world. Schelling called Bacon, Newton, and Boyle destroyers of astronomy and physics. The great Goethe (whose influence, according to Bünning, may still be felt in the publications of certain present day botanists, especially morphologists) characterized Newton's optics as being plain nonsense. The dominant influence of philosophy not only prevented experimental work being carried out but also prevented good experimental work from becoming known. Bünning has pointed out that when Mendel communicated his findings to the famous botanist Carl Wilhelm Nägeli (1817–1891), the latter—indoctrinated by Oken (1779–1851) and Hegel (1770–1831)—wrote back "... your results are only empirical data; nothing in them is rational". In the atmosphere prevailing then some biologists like Hans Driesch (1867–1941) gave up laboratory research and took to philosophy.

Erwin Bünning

Since academic positions were scarce in the 1930s, Bünning was happy to become a scientific assistant in the University of Jena in 1930. The botany tradition in Jena goes back to Goethe (1749–1832), Schleiden, Pringsheim, and Strassburger. The liberal geneticist Otto Renner was 'full professor'. The majority of students (but not professors) were Nazi supporters which made life difficult for Bünning. He later accepted a lectureship in the far-off University of Koenigsberg (Eastern Prussia, later Soviet Union). Bünning went away to Java and Sumatra in 1938 for a year and his experiences there are described in the book *Tropische Regenwälder*. Once back he was conscripted as a soldier in 1939. He wrote, "My interest in this profession becomes clear from the fact that I never reached the rank of an officer" but a soldier he had to remain until the end of the war, at which time he escaped becoming prisoner of war by hiking long distances through the cover of the Black Forest. Bünning became a professor in 1945 at the University of Cologne and later in 1946 at the University of Tübingen where he stayed till the end.

The botany institute in Tübingen was one of the few which were not destroyed in the war. It was built in 1846 under the direction of Hugo von Mohl. Part of the equipment was still from Wilhelm Pfeffer's time there (1878–1887). Bünning found in Tübingen a challenging tradition in botany. Here in 1694 Cammerarius discovered sexuality in plants. Correns rediscovered Mendel's Laws in 1900. Vöchting carried out his experiments on polarity and other aspects of developmental physiology. The ingenious Wilhelm Hofmeister, who was a book seller before he became a professor at the age of 39, also worked there.

Bünning had varied interests and was much more than a chronobiologist, even though his research publications, not to mention the instant classic which his book became, would seem to suggest otherwise. (As a point of some interest, the only abbreviations Bünning ever used in this book—written in a field that was getting jargon-ridden—were LL, LD and DD and there were no Greek alphabets or symbols at all.) He and his wife were well informed about India and its culture and the famous indologist Helmut von Glasenapp was their friend. He published over 260 papers in various fields of plant physiology and general biology and a very popular text book on plant physiology. He liked to be called a biologist and not a botanist. A travelogue he wrote in 1949 had the title *"In den Waldern Nordsumatras. Reisebuch eines Biologen"*. He wrote a delightful biography of his role model Wilhelm Pfeffer with whom he shared many character traits.

I recollect two important events from Bünning's laboratory in the mid-1960s. One was a seminar given by H G Schweiger on circadian rhythms in CO_2 output in the giant algal cell *Acetabularia* which apparently persisted after a portion containing the nucleus had been extirpated. This was some time in 1965 and was (then) sensational news. The most conspicuous methodological flaw was that Schweiger had taken only two readings per cycle i.e., a reading of CO_2 emission every 12 h. K Brinkmann and Claus Schilde led the onslaught and asked Schweiger what the rationale was. He said that the readings were difficult to take. Bünning sat through it all without a comment. The other seminar was by Charles Ehret about a molecular biological clock model (Ehret and Trucco model) also in 1965 which was the first model of its kind. Ehret was apparently discussing this in public for the first time (Ehret and Trucco 1967). Like truly great men Bünning was very modest and reluctant to talk about himself. In the last year of his life he had to be hospitalized. I had the final privilege in 1991 of giving a talk "Erwin Bünning: In Memoriam" at the Gordon Research Conference on Chronobiology in Irsee, Bavaria.

3.3 *Colin S Pittendrigh (1918–1996)*

The conceptual foundations of the field of biological rhythm research in this century were largely laid by Aschoff, Bünning and Pittendrigh. Pittendrigh brought to chronobiology a degree of experimental elegance and rigour which remained unsurpassed. He was born in Whitley Bay, Northumberland, in the north of England. His ancestors were Scottish/Gaelic. He secured the B.Sc.(Honours) degree with a First from the University of Durham, England. In high school he read *The Origin of Species* and later wrote, "My high school interest in Darwinian evolution survived an undergraduate exposure to J W Heslop-Harrison's Lamarckian convictions, flourished during graduate school (Columbia) with Dobzhansky, and matured during several later years of friendship and collaboration on a book with G G Simpson" (Pittendrigh 1993). During war service he was stationed in Trinidad during 1942–45 where he worked on the ecological basis and control of bromeliad malaria among the personnel of the Army, Navy and Air Force bases. He obtained his Ph.D. degree from Columbia University in 1946 and joined Princeton University in 1947 as assistant professor and became full professor in 1957.

Pittendrigh convened the famous meeting on Biological Clocks as the XXV Cold Spring Harbor Symposium in 1960 with Erwin Bünning in the chair. In this meeting Pittendrigh achieved many scientific objectives (Pittendrigh 1960). He drew pointed attention to Bünning's work and gave the name to Bünning's hypothesis. Aschoff had accumulated data over the years that the (free-running) period length of circadian rhythms of nocturnal animals was consistently shorter than the period length of diurnal animals (Aschoff 1960). Aschoff formulated this in an ecologically meaningful way and called it the Circadian Rule. Pittendrigh renamed it Aschoff's Rule and Aschoff's Rule it continues to be. Of the triumvirate Aschoff–Bünning–Pittendrigh, he was the youngest and the most charismatic. He was passionate in exposition and ruthless in exposing slip-shod science.

Pittendrigh wrote eminently readable scientific papers. Enormous experimental details and data went into his publications; he nearly never wrote anything trivial. He abhorred sloppiness in the work of others. He wrote early in one of his papers that the light pulse phase response curve (PRC) that he and V G Bruce had constructed for the eclosion rhythm of *Drosophila pseudoobscura* depicts the time course and wave form of the underlying oscillator. (The PRC set out steady state phase shifts as a function of the perturbed phase.) With this picturesque and evocative description, he raised the phase response curve of *Drosophila* to the status of a powerful tool which was then used by subsequent workers in formulating gedanken—and practical—experiments and in constructing predictive models to explain phenomena such as phase shifts, transients and entrainment.

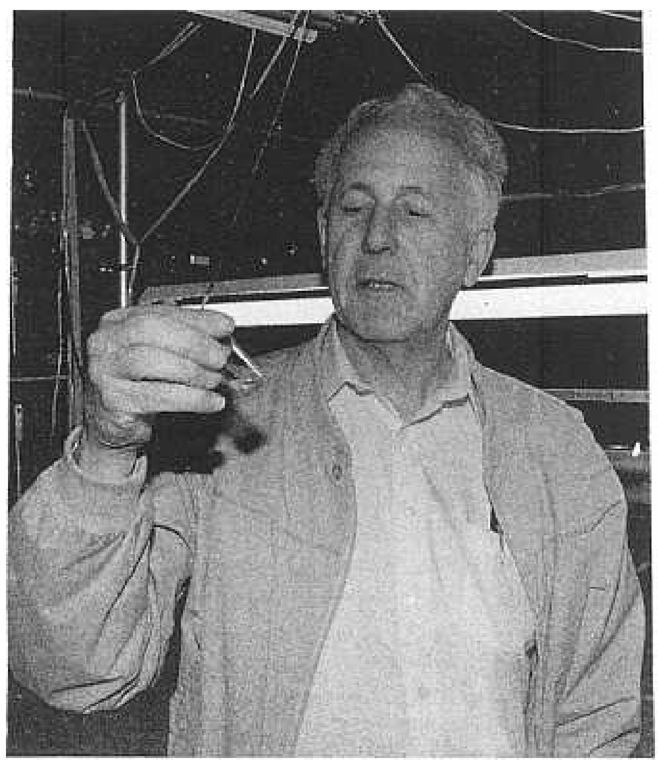
It is my recollection that Pittendrigh came to Calfornia in the winter of 1968 when he was toying with the idea of moving over to Stanford University and was invited to give a talk in Berkeley, where I then was. I showed him some *D. pseudoobscura* eclosion rhythm data of mine, which I was to publish the following year. He seemed to be genuinely excited, especially because my data supported the main tenet of his coupled oscillator model that brief light pulses phase-shifted the master, pace-maker oscillator instantaneously by magnitudes predicted in the light pulse PRC. The postulate was astonishing, original and picturesquely explained the phenomenon of transients, on which Pittendrigh and colleagues had written the only paper ever solely devoted to transients.

He often told me how much he admired Bünning but their meetings became rare in later years. The last important occasion which brought Aschoff, Bünning and Pittendrigh together was a seminar which Pittendrigh gave in two sessions in September 1971 at the Max-Planck-Institut in Andechs. He was at his eloquent best and spoke about his finding that whereas red light at wavelengths longer than 610 nm could photoperiodically cause diapause in the moth *Pectinophora gossypielle*, the same red light did not entrain the circadian rhythm in oviposition. Since the circadian rhythm is supposed to underlie photoperiodic phenomena as stated in the Bünning hypothesis, the findings appeared to Pittendrigh to go against the main tenet of the hypothesis. Bünning, who never defended his views and had not referred to the 'Bünning hypothesis' in print or conversation, felt that the *Pectinophora* results could be explained if the response to red light should turn out to be a direct response and not a photoperiodic response. After several attempts to make his point to Pittendrigh. Bünning appealed to Aschoff, who intervened and Bünning just about got to state his case.

Pittendrigh was a brilliant lecturer and could be very pugnacious in debate. He also had very strong likes and dislikes. He found it difficult to feign politeness to scientists whom he did not like. He was forced to share a table at dinner at an annual meeting of the International Society for Chronobiology somewhere in Europe with a famous chronobiologist for whom he had scant regard. Many of us at the same table had never experienced such tension at a dinner. The discomfiture of both scientists and the perpetrators of this faux pas was palpable. He was the doyen of biological rhythms research in the USA and built a very influential school of chronobiologists who are the leading lights in the field today.

3.4 *John Woodland Hastings*

John Hastings was born on 24 March 1927. He is the youngest among the pioneer chronobiologists featured in this essay. He obtained his B.A. degree from Swarthmore College and M.A. and Ph.D. from Princeton University in 1951, when both he and Pittendrigh were at the same university but still had not discovered biological clocks for themselves. Hastings got along famously with Colin



John Woodland Hastings

Pittendrigh and was his teaching assistant for a course on invertebrate biology. But he did his Ph.D. thesis work with E Newton Harvey on bioluminescence well before Pittendrigh got into the clocks problem. Hastings recollects that Pittendrigh was already incubating the biological clocks issue for he is supposed to have asked if Hastings could cite examples of oscillating enzyme systems. Since Hastings was then working on enzymes reacting with oxygen, where he had observed overshoot and undershoot features their discussions were lively and mutually stimulating.

After two years of postdoctoral research at Johns Hopkins (1951–53) working on firefly luciferase and then bacterial luminescence Hastings took a position on the faculty at Northwestern University in Evanston, Illinois and the Chairman who hired him was Frank Brown Jr. He knew little then how much the two would clash intellectually in the next few years to come. Hastings was intensely interested in isolating the luciferin and luciferase from a dinoflagellate, knowing that it was different from all other forms and carried out this work in the summer of 1955 in La Jolla. He was successful in isolating and characterizing the system and discovered that the luciferase was present during the night phase but not during the day. Experiments investigating the nature of the rhythm showed that it was persistent (circadian) and he was well on his way into biological rhythm research. Hasting's results, needless to say, clashed with the proposals and models being advanced by his departmental chairman Frank Brown. Brown over the next five years orchestrated a strategic retreat culminating in the Cold Spring Harbor Symposium of 1960 where he put forward his 'autophasing' hypothesis. Hastings had the temerity to refer to Brown's hypothesis at this conference as "phase (face) saving" hypothesis, which it obviously was in both terms. Hastings feels it was this important meeting and a NATO supported meeting in Feldafing (Germany) in 1964 organized by Aschoff (1965) which really consolidated chronobiology.

Hastings (along with Sweeney) (Sweeney and Hastings 1957) reported diurnal rhythms in the luminescence of *Gonyaulax polyedra* in 1957 and the first phase response curve in 1958 (Hastings and Sweeney 1958) much before PRCs had become analytical and predictive tools in investigations of light relations and the kinetics of circadian clocks. In 1957 (Hastings and Sweeney 1957), just three years after the phenomenon itself was discovered, he called attention to the possible mechanism of temperature independence of the *Gonyaulax* rhythm. It is from Hastings' laboratory that the first action spectra for two effects of light, one in lengthening the period and the other in shortening the period, and for phase shifting of the clock, were reported. In Cold Spring Harbor in 1960 Hastings read a paper 'Biochemical aspects of rhythms: Phase shifting by chemicals'—the first report of its kind (Hastings 1960). He then went on to make the *Gonyaulax* system a classic, demonstrating a complex of rhythms all tightly coupled to one another. His Department of Molecular and Cellular Biology at Harvard University is a very consistently active centre and on the many visits I have paid Hastings, there were scientists from all over the world. Hastings was Chairman of the very successful Gordon Conference on Chronobiology of 1991 held in Kloster Irsee in Bavaria, Germany. He now concentrates on the molecular genetics of the circadian organization in his and everyone else's favourite dinoflagellate—*G. polyedra*.

4. A pointless controversy

A controversy which arose in explaining persistent rhythms was whether they were endogenous in origin or were caused by unknown external factors. This continued into the middle of the 20th century. Having observed the sleep movements of leaves and how complicated they were, Darwin had written at length about the ability of plants and their leaves to show movements; but he remained mystified about their selective value. Those were the times when plants and animals were supposed to have continually responded with 'tropisms' to all manner of stimuli from the outside world. The successors of Jacques Loeb (1859–1924), who spread the message of tropisms assiduously, interpreted all plant, animal and even human behaviour as emerging from a chain of tropisms and inherited the nomenclature of 'behaviourists'.

Wilhelm Pfeffer also believed that the sleep movements of the leaves of plants occurred in response to unknown factors of exogenous origin. Early in his career he was convinced that these rhythms could not be innate. The sterile debate on the endogenous versus exogenous origin of biological rhythms persisted right into the 1980s. As late as 1987, a publication carried the title 'Circadian rhythms—endogenous or exogenous?' (Brady 1987) and went on to demonstrate that it was unnecessary to reopen the topic. One could not understand why some of the proponents of the exogenous origin of rhythms continued to hold to their views in the face of incontrovertible evidence to the contrary. In the course of his later extensive experiments Pfeffer gradually revised several of his original ideas and came to accept the endogenous, circadian nature of the sleep movement rhythms of plants. He expressed these views on diurnal leaf movements in a series of papers that filled about 750 printed pages. A brief review in the form of original passages translated into English has been published (Bünning and Chandrashekaran 1975). A particularly resilient champion of the exogenous viewpoint was Frank A Brown Jr who was asked by the Encyclopedia Britannica to write on the topic of biological clocks. He was a Harvard-trained invertebrate endocrinologist and had worked on the hormones of crustacea before coming to biological rhythms some time in the 1940s. He and his students were prolific but hardly ever mentioned earlier work of European origin in their papers. In 1998 Patricia DeCoursey, who had known Brown well, told me that his co-workers adored him and hung on uncritically to every pronouncement he made.

The real danger was that in the 1940s and 1950s Brown was the high priest of the subject and forcefully propagated his message. Even though he had not established a school (as had Aschoff in Andechs, Bünning at Tübingen and Pittendrigh at Princeton and Stanford) he had followers. Support for his postulate of 'subtle geophysical factors' arising as a consequence of the rotation of the earth on its own axis, came from unexpected quarters as, for instance later, from bee researchers (Brady 1987). Brown expounded tirelessly how electrostatic and magnetic field variations provided organisms 24 h cues even under supposedly constant conditions. He made the mistake, however, of presenting his views in the Cold Spring Harbor Symposium of 1960 (Brown 1960). Pittendrigh, who had brought to the field a degree of elegance and rigour, was just then in top form. At the symposium the question of the endogenous versus exogenous origin of circadian rhythms was discussed at length for the last time. The scales of scientific authenticity were steeply tilted in favour of an endogenous (genetic) origin. Yet Brown rashly stated in discussion that his opponents, in insisting upon a self-timed or fully autonomous clock, were faced with the possibility that they were pursuing a ghost. To this Pittendrigh retorted, "The question of the ghost is simple—either it is an aspect of living organization, or an unknown geophysical variable. My taste in ghosts suggests the latter but, as scientist, I must agree Dr Brown may prove right, and as scientist he will doubtless agree he may prove wrong. We both will have some fun in any case". Thereafter Brown fought a lone and losing battle; the waning of his influence in chronobiology had already started in the late 1950s. Using Brown's own controversial statistical methods, Cole (1957) demonstrated a strictly 24 h biological clock in that mythical animal, the unicorn! In November 1979 in a chronobiology meeting organized by Heinz von Mayersbach in Hanover, and attended by, among others, Aschoff and Bünning, Brown gave a talk in which he seriously claimed that two plants kept in two separate environmental chambers in close proximity, influenced the circadian rhythms of each other. It was a rambling talk delivered in a low key. There were no questions or comments from the audience. Even the normally loquacious Aschoff did not utter a word.

Brown was also at times lax with his observations and words. He thus wrote as late as in 1974 that, "Viewing the results of his numerous experiments with plant circadian rhythms, Pfeffer concluded that his organisms must each have their own independent internal clock. The conclusion was not compelled by the evidence but simply chosen as the preferred hypothesis" (Brown 1974). But, as Bünning pointed out in a letter, the exact opposite was the case. Later Bünning and Chandrashekaran (1975) commented: "Prof. Brown has confirmed in a letter that his misrepresentation of Pfeffer's views is the result of his insufficient knowledge of the German language and, consequently, of Pfeffer's publications".

5. What is in a name?

Resistance to scientific ideas is not always due to lack of communication resulting from language barriers. Resistance can also arise out of preferred bias. And because of calling things by the wrong name. The story of circadian rhythms in bees is instructive here. Beling (1929), a student of Karl von Frisch, had strong evidence in 1929 that his bees were orienting in time with the help of endogenous clocks. Time training experiments in which food was daily offered at a given hour, had to be 24 h apart and the insects would not (en)train to 19 h cycles. Three years later, Otto Wahl demonstrated that the rhythm, which the members of the von Frisch school called variously *Zeitsinn* (time sense) or *Zeitgedächtnis* (time memory), was inborn not learnt, and that honey bees that had never experienced light : dark cycles could still be entrained to 24 h cycles of restricted feeding in total darkness. There was a great reluctance to call the phenomenon by its right name of circadian rhythm. This cannot be seen in retrospect as an eccentricity or as a harmless fad. Having called it *Zeitsinn* the bee researchers began concentrating on environmental parameters such as the sun's azimuth, polarization of light, landmarks, humidity, wind direction and wind speed as possible causes, to the detriment of progress in rhythm research. Renner (1955) later performed the by now famous translocation experiments on honey bees, training them to search for food in Paris and flying them to New York, to see if they relied on endogenous time keeping or responded to local (New York) time cues. Pittendrigh (1993) commented: "While in Munich in 1959, I asked Martin Lindauer why they (the von Frisch laboratory) had still not reset the bee's clock with a Hoffmann-like shift of the light : dark cycle" (referring to K Hoffmann's demonstration that a time-shift in the light : dark cycle caused a proportional shift in the orientational clock in starlings). "Because", he replied "bees don't have clocks, they have *Zeitgedächtnis*"! That is what is in a name. That is also why so few people outside Germany knew about the monumental contributions of these early workers to chronobiology.

There are calendars sold now in supermarkets which inform the owner of his good and bad days, the days on which some things may be done, and days on which certain things may not be done. This pseudoscience called 'biorhythms' threatened for a while the newly won respectability of chronobiology of the biological rhythms kind. Believers in the cult of biorhythms proclaim the existence of the 23-day male cycle, 28-day female cycle, 33-day intellectual cycle, a 38-day compassion cycle, a 43-day aesthetic cycle, a 48-day self-awareness cycle and 53-day spiritual cycle. An author of a book on this subject (Gittelson 1983) states that Dr Wilhelm Fliess of Berlin started it all in 1880. This Fliess was an otolaryngologist, a member of the Berlin Board of Health, President of the German Academy of Sciences and a friend and mentor of Sigmund Freud. There is no evidence that Fliess impressed even his contemporaries with his notions regarding biorhythms. By the time of his death in 1928 he was yet to see biorhythms accepted as any kind of science in his native land, let alone elsewhere. As for a modern opinion on biorhythms here is the trenchant comment of Pittendrigh: "I consider this stuff an utter, total and unadulterated fraud. I consider anyone who offers to explain my life in terms of 23-day rhythms a numerological nut, just like somebody who wants to explore the rhythms of pig-iron prices to 14 decimal places". A National Institute of Mental Health (USA) paper classifies biorhythms as mythology. With all its absurdities, the concept of biorhythms has an obvious attraction for the sort of people who are drawn to parapsychology, astrology, horoscopes and to a belief in UFOs (Gittelson 1983).

6. Pacemakers, hourglasses and oscillators

An early researcher who caught my undergraduate imagination was Janet Harker of Cambridge, UK. From 1954 to 1956, Harker published papers in British journals on factors influencing diurnal rhythms in the locomotor activity of the cockroach *Periplaneta americana* (Harker 1954, 1956). She had been the first to show the participation of the endocrine system in the locomotor activity of an insect. She also reported results of exciting experiments in which cockroaches were made arhythmic in continuous light and then joined back-to-back in parabiosis with rhythmic cockroaches. The arhythmic member of the pair was mobile but the legs of the upper rhythmic member were removed. The locomotor activity of the mobile animal was then said to follow the circadian rhythm of the upper cockroach. She maintained that the pacemaking oscillator in the roach was housed in the sub-oesophageal gland. If she cooled the organ *in situ* for 4 h the clock was delayed for 4 h at all phases. Enthusiastic efforts by Shephard Roberts, a Ph.D. student of Pittendrigh at Princeton, failed to confirm Harker's findings. The uneasy relationship between Harker and Pittendrigh is reflected in the discussion that followed her presentation in the Cold Spring Harbor Symposium of 1960. In her 1960 paper (Harker 1960) Harker referred to the work of just two other authors on biological rhythms; oddly, she did not cite her own review of 1958, among the earliest on diurnal rhythms (Harker 1958). She did not straightaway endear herself to Pittendrigh by posing the question: can one can speak of a biological clock when one worked on eclosion rhythms in populations of pupae (as Pittendrigh did)? This was an unnecessary caveat since Pittendrigh had beautifully demonstrated the precision of the process of gating of the eclosion rhythm in *D. pseudoobscura*, which showed that the rhythms in individual flies were in synchrony. She also believed that her 4 h of delay at all phases of the rhythm of the roach was an "argument against regarding the rhythm as being dependent on a relaxation oscillator". Many of Harkers findings have not been confirmed even though research on cockroach rhythms has flourished and is still in progress. Later Nishiitsutsuji-Uwo and Pittendrigh (1968) reported that the locus of the driving oscillation might be the optic lobe, a finding that has since been repeatedly confirmed.

The names of F A Brown Jr and J Harker were hardly ever mentioned even in passing in the weekly seminars of Bünning's Botanisches Institut in Wilhelmstrasse in the period 1964–67 when I worked there. In fact even the views of A D Lees, who had demonstrated for the aphid *Megoura viciae* that the photoperiodic time measurement was being mediated by something much like an "hourglass", were seldom critically discussed in Tübingen. This could have been the result of Bünning's own attitude towards controversies, to steer clear of them. Like Medawar he preferred to avoid controversies of any kind. The organisers of an international meeting on Circadian Rhythmicity at Wageningen had invited both Lees and Bünning and were hoping there would be animated discussion between those who subscribed to the 'hourglass' hypothesis and the others who subscribed to the 'endogenous oscillations' hypothesis. Lees spoke reverentially of Professor Bünning's hypothesis but concluded that the aphids may be in a separate class in themselves. Bünning had earlier given the chairman's address and sat impassively in the front row. The chairman of this session, de Wilde, asked, "Does Professor Bünning have anything to say?". Bünning replied "I have extensively written and spoken about the role of circadian rhythms in photoperiodism. As for Lees' findings, I believe them, and nature has certainly more ways than just one, to solve problems." Interestingly, it was Lees who displayed a slide of the Bünning model—not Bünning—showing the phase relationships of the hypothetical rhythm of light sensitivity under long and short day conditions (Lees 1971).

7. The clock gene and after

On one winter evening in Berkeley in 1968, Pittendrigh suddenly surprised me with the statement: "Once we succeed in finding the clock gene ... boy, we'd lick the problem of mechanisms..." (of circadian rhythms). His musings made it sound as though the eventuality were distant and wistful. A year later, in his laboratory Ron Konopka, a student of Seymour Benzer, hit upon the clock gene in *Drosophila melanogaster*. The formal paper with details was published soon after (Konopka and Benzer 1971). Biological rhythms research was now entering the new age. But it is proper to remember that the first evidence for the genetic basis of circadian rhythms was provided by Bünning (1932). 'Clock genes' have now been reported in the bread mould *Neurospora*, *Arabidopsis* and cyanobacteria. There was much rejoicing at the identification and cloning of the first clock gene in mammals—the mouse—by the researchers in the Center for Biological Timing at Northwestern University, Chicago, led by Joseph Takahashi (Antoch *et al* 1997). This, a landmark event, has been hailed as a nugget of circadian gold. Because of the known relationships between the *Mus* and *Homo* genomes, it is only a matter of time until someone identifies the homologous gene in humans.

Joseph S Takahashi

The importance of circadian rhythms in human biology is impressive. They are directly or indirectly implicated in variations in hormonal levels, pharmacokinetics, timing of heart attacks, intensity of asthma, jet lag, adjustments to shift work, sleep disorders and seasonal affective disorders (winter blues). Barely 20 years ago it was erroneously believed (Wever 1979) that human circadian rhythms, unlike those in other animals, were impervious to exposure to light and darkness. It was later discovered that this misconception arose since earlier work had employed light intensities much below the threshold for entrainment. A paper published this year (Campbell and Murphy 1998) challenges the widely held belief that mammals are incapable of extraretinal circadian photo-transduction. The authors showed that circadian rhythms in body temperature and melatonin concentration in humans could be phase shifted by light pulses presented to the popliteal region (behind the knee).

Just as the Cold Spring Harbor Symposium of 1960 on Biological Clocks gave chronobiology respectability, the convening of the Gordon Research Conferences on Chronobiology every two years since 1981 in Europe or the USA has further invigorated the field. Also, a Society for Research on Biological Rhythms meets every year and there are several national societies that deal with the subject area; there is even a Society for Light Treatment and Biological Rhythms for the treatment of seasonal affective disorders. It is heartening for chrono-biologists that their subject has made the transition from the presumed status of metaphysics to the high altar of molecular biology. My personal opinion is that for those of us who have always been fascinated by the myriad behavioural expressions of biological clocks, the exciting era has just started. There are many vital questions that remain to be answered. For example, the relationship between the family of lunar (or tidal) rhythms and circadian clocks remains to be elucidated. One is not even sure of the exact nature of the entraining agents ('Zeitgebers') responsible for the lunar rhythms; and what the molecular biology of circannual rhythms might be, remains entirely obscure.

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