LIVING CLOCKS IN THE ANIMAL WORLD

MIRIAM F. BENNETT, Ph.D.
Chairman and Professor of Biology
Colby College
Waterville, Maine

Publication Number 902
AMERICAN LECTURE SERIES

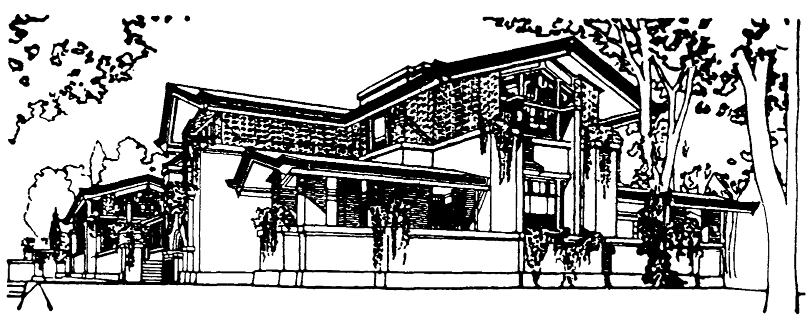
A Monograph in
AMERICAN LECTURES IN ENVIRONMENTAL STUDIES

Edited by
CHARLES G. WILBER
Professor of Zoology
Colorado State University
Fort Collins, Colorado

Living Clocks in the Animal World

By

MIRIAM F. BENNETT, Ph.D.
Chairman and Professor of Biology
Colby College
Waterville, Maine



CHARLES C THOMAS • PUBLISHER
Springfield • Illinois • U.S.A.

Published and Distributed Throughout the World by
CHARLES C THOMAS • PUBLISHER
Bannerstone House
301-327 East Lawrence Avenue, Springfield, Illinois, U.S.A.

This book is protected by copyright. No part of it
may be reproduced in any manner without written
permission from the publisher

©*1974, by* CHARLES C THOMAS • PUBLISHER
ISBN 0-398-02872-9
Library of Congress Catalog Card Number: 73-5573

With THOMAS BOOKS careful attention is given to all details of manufacturing and design. It is the Publisher's desire to present books that are satisfactory as to their physical qualities and artistic possibilities and appropriate for their particular use. THOMAS BOOKS will be true to those laws of quality that assure a good name and good will.

Printed in the United States of America
I-1

Library of Congress Cataloging in Publication Data
Bennett, Miriam F
Living clocks in the animal world.
(American lecture series, publication no. 902. A
monograph in American lectures in environmental studies)
Bibliography: p.
1. Biology—Periodicity. I. Title.
QH527.B46 591.1 73-5573
ISBN 0-398-02872-9

For Peter and Andrew

FOREWORD

THIS FIRST VOLUME in the American Lecture Series on Environmental Studies is timely and appropriate. The study of biorhythms in animals (animal clocks) is a basic biological endeavor with important applied aspects.

Plants and animals show a distinct diurnal rhythm in their various activities. Cues from outside the organisms may force them into precise cycles. Invertebrate animals, lower vertebrates, mammals, subhuman primates and man exhibit a daily patterned cycle of rest and activity. This daily rhythm is called circadian (Latin "circa diem," about a day).

In many, there is a daily regular pulsation of body temperature, blood pressure, pulse rate, respiration, hemoglobin levels, and blood amino acid concentration.

Man lives on planet Earth which is subject to various rhythms – lunar, solar, seasonal. As a general rule, these natural rhythms are ignored by man in his social planning.

Recent studies in human biology show that "biological time of day appears to be hugely important in physiology. Time structure in the body may be almost as important as tissue."

Scheduling may well be a crucial factor in human well-being. What threat is posed by disrupting the deep-seated human circadian rhythm? We are not sure. It is certain that each species of animal does have a built-in rhythm of various functions. The rhythms depend on external (environmental) clues. It is long overdue that planning for human health and welfare acknowledge these inborn clocks as critical factors for *Homo sapiens*.

A recent study of nearly 2000 homicides in Dade County, Florida has shown that the murder rate "rose the day before a full moon, reached a peak at full moon and dropped back before a second peak at the new moon."[1] Virtually 90 percent of the homicides were committed during a full moon.

[1]Anon.: Murder and the moon. *The State Peace Officers Journal, 5*: 28-29, 1973.

Similar lunar influences were found for homicides in Cuyahoga County, Ohio. Internal "biological tides" in the human body have been suggested as possible violence triggering mechanisms.

Professor Bennett's book provides the basic biological data for a clearer understanding of the significance of biorhythms.

CHARLES G. WILBER

PREFACE

DURING THE LAST TWELVE YEARS, the reports of papers presented during several international conferences on biological timing have been published: The Cold Spring Harbor Symposium on *Biological Clocks* (1960), the Feldafing Summer School on *Circadian Clocks* (1965), the Friday Harbor Symposium on *Biochronometry* (1971), the Tihany Symposium on *Invertebrate Neurobiology: Mechanisms of Rhythm Regulation* (1973) and the International Society for the Study of Biological Rhythms meeting on *Chronobiology* (1973). Additionally, books focused on specific areas of biological timing are available. Among these are: Beck's *Insect Photoperiodism* (1968), Bünning's *The Physiological Clock* (1967), Cloudsley-Thompson's *Rhythmic Activity in Animal Physiology and Behaviour* (1961), Conroy's and Mill's *Human Circadian Rhythms* (1970), Harker's *The Physiology of Diurnal Rhythms* (1964), Palmer's *The Biological Clock. Two Views* (1970) and Sweeney's *Rhythmic Phenomena in Plants* (1969). Some of these publications as well as many studies of living clocks – both of the past and of the present – are pointed up in this book. I am convinced that familiarity with the literature and the investigators of any field of inquiry is demanded of its practitioners and is enjoyed by most of its spectators.

In this book, I have not attempted to review our knowledge of biological rhythms, in general, or that of timing mechanisms in large segments of the living world. The authors cited above – at least *in toto* – have done that and have done it well. Rather, I have tried to focus on the chronometry of particular animals – forms with which I have had some direct experience. I have attempted to underline the contributions of those animals to our fund of information about biological cycles. What have we learned from specific studies of specific animals? What major questions about their timing phenomena remain? How can we best attack those problems in our attempt to analyze, to synthesize and to understand the rhythms of those species and the rhythms of the living world as a whole?

ACKNOWLEDGMENTS

AS A GRADUATE STUDENT, I was introduced to animals' clocks by Professor Frank A. Brown, Jr. of Northwestern University. My thanks go to him for his guidance of my early work and for his interest in my studies of organismic timing which I continued at Sweet Briar College. I am also grateful to Professors Hansjochum Autrum and Maximillian Renner of the Institute of Zoology of the University of Munich, for the hospitality and aid afforded me during long-term stays in that institute.

I am especially happy to thank many of my students at Sweet Briar College – Dana C. Reinschmidt, Mary-Fleming Willis Finlay, Judith A. Harbottle, Carolyn B. Guilford, Jan Huguenin, Charlene Reed and Joan H. Spisso – for their technical assistance, discussions, arguments, criticisms and advice which have contributed immeasurably to my studies of animals' clocks, to my understanding of the temporal behavior of animals and to my delight in learning and teaching about living clocks.

It is also a great pleasure to acknowledge the contributions of Dean Catherine S. Sims of Sweet Briar College who encouraged me to write this book and who aided the project in many, many ways. Financial help for my own studies and for the preparation of the book has come from the Office of Naval Research, the National Science Foundation, the Sigma Xi Club of Lynchburg, Virginia, the Kampmann Award of the Sweet Briar Alumnae Association, the Committee on Faculty Research, Sweet Briar College and the Anonymous Donor's Science Fund of Sweet Briar College.

The persons who read and corrected my manuscript – in particular Naomi B. Erdmann and Dorothy Vickery – deserve many thanks. But, they share no blame for errors or points of confusion. Those are all mine. I also wish to thank Julia S. Child and artists of the Frank Wright Studio of Lynchburg, Virginia for their drawings and Cecille Harvey for her typing.

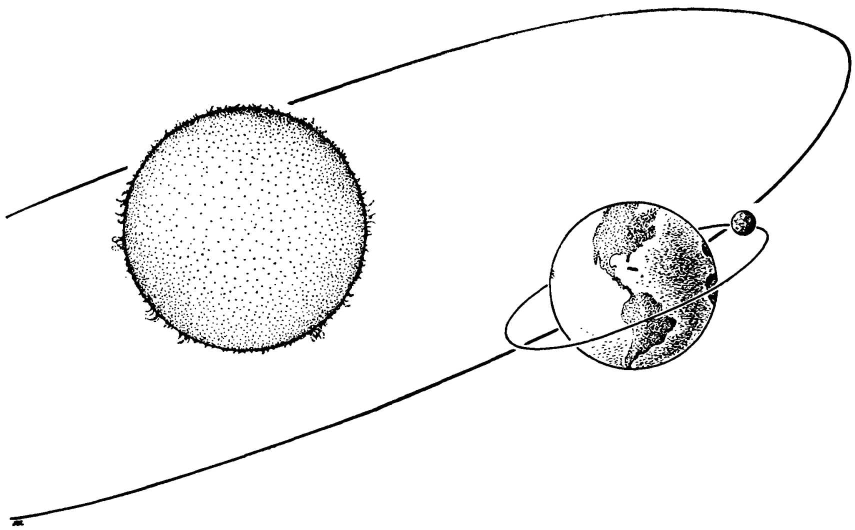
Authors whose figures have been used are cited in the legends. My thanks go to them and to the following for permission to publish illustrations which have been copyrighted:

Academic Press, Inc., New York
American Association for the Advancement of Science, Washington
The American Midland Naturalist, Notre Dame
American Physiological Society, Bethesda
The Biological Bulletin, Woods Hole
Cold Spring Harbor Laboratory, Cold Spring Harbor
Cornell University Press, Ithaca
Macmillan Journals, Ltd., London
The National Academy of Sciences, Washington
North-Holland Publishing Company, Amsterdam
Springer-Verlag, Berlin-Heidelberg-New York
The Society for Experimental Biology and Medicine, Utica
The University of Chicago Press, Chicago
The Wistar Institute of Anatomy and Biology, Philadelphia

July, 1973 Miriam F. Bennett

CONTENTS

LIVING CLOCKS IN THE ANIMAL WORLD



Chapter 1

LIVING CLOCKS PAST AND PRESENT

LOREN EISELEY, himself very much a student of life in terms of time, believes ours to be ". . . the most time-conscious generation that has ever lived." (Eiseley, 1969, p. 5). We have come by this reputation simply and honestly, for we merely reflect the billions of years of evolution of biological timing that have come before us. Time-consciousness is undoubtedly an attribute only of man, but having the capacity to live in time with changes of the sun and the moon, the tides and the seasons is characteristic of all life on this earth, and probably has been so since that life came into being.

Man's realization that he is not the only timekeeping species is ancient. Androsthenes, an officer who accompanied Alexander the Great on his explorations, observed the daytime rising and the nighttime falling of the leaves of the tropical Tamarind tree. Similar notes about changing positions of leaves are found in the first century writings of Pliny, the Elder, and in the thirteenth century writings of Albertus Magnus. These changes are now referred to as "sleep movements," for in the seventeenth century, Linnaeus described the nocturnal positions of leaves as plant sleep. In the eighteenth century, De Mairan, an astronomer most interested in the rotation of the earth, and the botanists, Duhamel and Zinn, were able to report that changes in light intensity of the plants' surroundings did not cause the sleep movements of the leaves, since their rising and falling continued for some period of time even when the plants were kept in constant darkness or under constant artificial light. European botanists continued to lead as investigators of biological timing during the nineteenth century when they were joined by Charles Darwin, who described temporal changes in clover and wood sorrel as well as in earthworms, and by the Viennese zoologist, Kiesel, who recorded day-night changes in the retinal pigments of arthropods.

So, by the beginning of the twentieth century, examples of both plant and animal clocks had been recorded in the scientific literature. What are these biological or living clocks? Biological clocks are inherited, cellular mechanisms with which organisms mark off or measure the passage of time cyclically. The ticking or beating of such precisely integrated collections of molecules – whatever they may be – allows living systems to vary the intensity or the level of many diverse functions in regularly repetitive fashions. The rhythmically recurring changes, *e.g.*, the positions of the leaves of plants, the positions of the pigments of the eyes of shrimp, the rates of crawling of earthworms, which are regulated by the clock, *but are not identical with it,* continue for some time even when the organisms are removed from their natural environments, and are maintained under laboratory conditions of constant light intensity, constant temperature and constant humidity. The frequencies of those changes or the periods of time between the occurrence of similar events or levels of the cycles under the constant conditions are very close to those measured while the organisms are exposed to changes in natural environmental factors such as light and temperature which occur fairly regularly in their real worlds. Therefore, one can state that by virtue of cellular clocks, rhythms of some organismic activities persist under the unvarying and thereby, the atypical "constant conditions" of the laboratory.

The present-day student of the clocks of animals should stop briefly during a rapid review of the history of studies of biological timing to consider the investigations of Pfeffer, much of whose work was on the clocks of plants, especially of beans and marigolds. At first, he doubted the validity of claims of the continuance or persistence of sleep movements under constant conditions. He thought that perhaps light which had leaked into caves and darkrooms used for the eighteenth century experiments had induced the daytime positions of the leaves. So, he repeated some of those experiments. After his own observations supported the findings of Duhamel and Zinn, he, also, was convinced of the reality of biological changes which persisted at frequencies similar to those of changes in the plants' normal outside worlds even when the organisms were in the laboratory and were deprived of physical variations, *e.g.*, light to dark, which might function in the natural environments of the plants as temporal cues or time signals.

An endogenous component of organisms' 24-hour cycles was therefore recognized and emphasized by the early twentieth century, for Pfeffer concluded that the frequencies of plant rhythms depended upon an internal, organic mechanism. This mechanism, independent of environmental factors, was believed to generate the periods or durations of persistent periodic phenomena, other aspects of which, amplitude and phasing, were influenced and regulated by changes in the physical surroundings of the plants. Here, basically, is the view of the nature of persistent biological rhythms of plants, animals and microorganisms held by most students of biochronometry today.

The second view of the nature of the basis of organic timing was also espoused in the early twentieth century, again by a botanist, Stoppel. She and her present-day supporters believe that living clocks demand information from their physical environments to be able to generate the frequencies of their cyclic activities. Even under "constant laboratory conditions" subtle, cyclic geophysical factors are not screened out and therefore are available to organisms as time cues. According to this hypothesis, physical factors not only affect the amplitude and form of persistent rhythms, but they are also necessary for the maintenance of the lengths or periods of the cycles. Pfeffer, Stoppel, their forerunners and their contemporaries did indeed lay the foundation for the newer attacks on biological clocks which attracted the attentions of more and more biologists of many different specialties during the 1930's. That attraction has increased, and in terms of the number of investigators active in biochronometry, ours certainly is the most time-conscious generation of biologists that has ever lived.

Erwin Bünning of the University of Tübingen whose own writings (1960 and 1967) include excellent historical surveys of the study of biological clocks, is one of this century's leaders in the field. His earlier work focused on rhythms of both plants and animals, although that of more recent years has concerned only plants. His analyses of the effects of temperature and light changes on cycles are outstanding. His emphasis on the relationship between persistent rhythms and photoperiodic effects have stimulated significant recent studies (*e.g.*, Pittendrigh, 1963). Bünning's hypotheses of the oscillatory nature of the internal timing mechanism have prompted the development of models, mathematical theories and engineering parallels which attempt to explain the basic workings of living clocks. Observations and experimental results from biological laboratories all over the world confirm and extend many of his original ideas.

Before the Second World War, Hans Kalmus of the University of London, Karl von Frisch of the University of Munich and the late Orlando Park of Northwestern University were leading the zoologists in their observations and analyses of animal clocks and of the ecological and behavioral significance such timing mechanisms have for their possessors. Curt Richter of the Johns Hopkins University, Nathaniel Kleitman of the University of Chicago and Franz Halberg, now at the University of Minnesota, were among the first to investigate precisely, periodic changes in human beings and to emphasize their importance in medicine and its practice. The reports of an intensive study of living clocks by Frank Brown and his students at Northwestern University and the Marine Biological Laboratory at Woods Hole, Massachusetts, began in 1948. That program continues, and as is to be seen in later chapters of this book, its publications contain records of some of the most amazing, provocative and enigmatic aspects of organismic timing. Brown now leads the school of investigators who believe, as did Stoppel, that cyclic, subtle geophysical factors are used by organisms to time their persistent rhythms.

During the 1950's the late Gustav Kramer of the Max Planck Institute in Wilhelmshaven, and von Frisch proved that animals so different as starlings and honey bees are able to move to the right place at the right time because of an intricate association of spatial and temporal behavioral patterns. Their works gave rise to the concept of clock-compass reactions or time-compensated orientation. In the same years, Jürgen Aschoff of the Max Planck Institute in Erling-Andechs and Colin Pittendrigh, now at Stanford University, came into prominence. Between them and their two groups of students and associates, they have probably studied physiological clocks in every major type of living system — microorganism, plant and animal. Their experiments have been beautifully conceived and executed. Their analyses of many parameters of rhythmicity and their variations are fundamental to biochronometry. Aschoff and Pittendrigh with Bünning lead the present-day adherents of the endogenous theory of biological timing, the idea supported by Pfeffer in the nineteenth century.

Yet now — in the second half of the twentieth century, most of the basic and fascinating questions and problems regarding living clocks remain. Their solutions will demand the continued enthusiasm and energy of a great many time-conscious investigators of our generation and of the generations to come. We must learn the actual structures and workings of biological clocks. What are their components? How do they function? How are they identified? What relationships do the clocks and their parts have to the physical environment of our earth? Are they adaptive to life on this planet? What relevance may they and knowledge of them have for us, a time-bound species? Partial answers to many of these questions have come to us from our work on animal clocks. However, a general discussion of the questions and answers which we have from studies of many forms of life is appropriate and necessary at this point to illustrate and to emphasize the principles, the theories and the vocabulary with which the student of biochronometry works.

That living clocks are cellular in nature has already been stated. Granted, only a part of the molecular population of cells or groups of cells may actually be engaged in generating the temporal outputs of living timepieces, but those molecules are parts of cells. To date, subcellular or acellular organic systems have not been shown to function as biological clocks. It has also been pointed out that the rhythmic processes, the crawling of earthworms and the sleep movements of leaves, which are regulated by the living clocks, persist in time with physical changes in the worms' or trees' natural habitats even after the organisms have been placed under "constant conditions" in the laboratory.

The periods, or their reciprocals, the frequencies, of the persistent rhythms are basically the same as those of the geophysical cycles of our surroundings. And these cycles, of course, depend upon the relative positions and movements of our earth, our moon and our sun. The majority of the persistent organismic cycles known and well studied are 24 hours in duration – the time of one rotation of the earth on its axis. These are usually called solar-day or circadian rhythms, but some authors continue to refer to them as diurnal rhythms. Cycles which recur at 12.4-hour intervals are tidal or primary lunar ones, while those of 24.8 hours are lunar-day rhythms. The frequencies of both these types of lunar cycles are the same as those of cycles of the environment which reflect movements of the earth relative to the moon, and those of the great air and water masses of the earth which are caused by the position of earth *vis a vis* moon. Lunar-monthly or 29.75-day rhythms have also been well documented for many organisms. A monthly rhythm may be the consequence of the organisms' simultaneous functioning at solar- and lunar-day frequencies. Lunar-monthly rhythms allow organisms to live in time with the orbiting of the moon around the earth. Least familiar and least studied are persistent annual cycles, those of approximately a year's duration, the time of the revolution of the earth around the sun. However, a few examples of yearly cycles of living systems have been recorded, and some are to be discussed in this book.

In the nineteenth century, De Candolle, a French botanist who investigated the sleep movements of *Mimosa,* clover and bean plants, noted the periods of some of those cycles to be a bit shorter than 24 hours. We now emphasize that the periods of most organismic rhythms expressed under constant laboratory conditions are very close to, but not identical with, the periods of geophysical cycles. Such periods or frequencies are the free-running ones, *i.e.*, they are seen in organisms living under the so-called constant laboratory conditions where no obvious time signals are conveyed by observational or experimental procedures. Free-running periods may be, and often are, longer or shorter than are the periods of the organismic cycles when the plants or animals are cued or are entrained by regularly repeated changes in their surroundings – either natural or experimental.

Thus, under free-running conditions, the persistent rhythms often run slightly out of phase with the actual day-night, lunar and annual rhythms of the earth. However, the periods of the cycles induced by living clocks vary within only narrow limits, *e.g.*, roughly 19 to 30 hours for circadian or solar cycles, and in a beautifully precise and highly adaptive manner can be attuned to the rhythms of the natural world by common and reliable changes of our normally varying physical environment.

Thus far I have focused upon the cellular timing mechanism, the living clock itself, but in doing so I have, necessarily, referred also to its hands, its indicators or the periodic organismic changes which persist under constant conditions. Again, one finds a European botanist of the nineteenth century, Sachs, to be the first investigator to emphasize this principle of biochronometry: living clocks and their hands are not identical with one another. He pointed out that periodic leaf movements, or the hands of the clocks of some plants, are dependent upon the periodicity of an "entire complex of processes." Sach's "entire complex of processes" may be equated with the cellular clock. It is imperative that we always distinguish between the clock and the hands, for their functions and characteristics vary, and several different sets of hands may be attached to the same set of clockworks. The common fiddler crab of our Atlantic shores shows persistent rhythms of both color change (Chapter 2) and locomotor activity (Chapter 3). One set of hands of its clock is color change; the second indicator of the clock is locomotor activity. Additionally, the anatomical and physiological attachments between the cellular timing mechanism and its indicators must be considered. Those tie-ups are often called mediating or regulatory pathways or transmission systems. In the fiddler crab, the mediating pathways between its biological clock and color change are primarily hormonal, while regulation of its cycles of locomotion depends upon the crab's nervous system. A variety of specific indicators and their regulatory systems are to be described and discussed in later chapters of this book.

And, almost chapter by chapter, these questions will arise: what is the true nature of living clocks and how do they function? As has been and will be stated repeatedly, we can not describe either the detailed anatomy or physiology of the clocks. We do not know exactly what they are or how they act. In an excellent recent review, *The Biological Clock. Two Views.* (Palmer, 1970), Frank Brown and J. W. Hastings each states and defends one of the two views. Brown deals with the hypothesis of environmental timing of the clock. Hastings concerns himself with that of a purely endogenous and independent clock. Both views embrace a physicochemical cellular mechanism which, according to Hastings, is fully autonomous, and which, according to Brown, must have informational input from the physical environment to be able to maintain its average precision and basic functioning. That input consists of fluctuations of geophysical factors of the earth and its sphere of influence which permeate the "constant conditions" of the laboratory, and repeat at solar and lunar frequencies. The organism "locks" its clock to those regular changes. What the factors are is also unknown, but geomagnetism and particular components of background radiation are leading candidates.

Naturally, any valid theory of the nature of living clocks must explain all characteristics of the basic timing mechanisms including two of their most amazing physiological ones: virtual temperature-independence or temperature-compensation through a wide range and insensitivity to chemical disruptions as great as some effected by cyanide, antibiotics and metabolic hormones. Ever since attacks on animal clocks began, investigators have marveled at the capacity of the cellular clocks to maintain their usual frequencies between approximately 5° and 30° C. They are neither slowed down by cold nor speeded up by heat, deviating, in those respects, from many biochemical systems which do have temperature coefficients of 2.0 to 4.0. For a 10° C rise in temperature, the rates of most chemical reactions are doubled, tripled or quadrupled.

The temperature-independence of living clocks remains one of our major enigmas. Will it be explained on the basis of a biochemical temperature-compensatory system, of an inherent property of biochemical oscillators, or as a natural consequence of the clock's being regulated by fluctuating geophysical factors whose effects are the same at 5° C as at 30° C? And how can the cellular clocks beat out their normal frequencies when vital metabolic steps, *e.g.*, enzyme or energy production, are inhibited or speeded up by chemical agents? Is absolutely balanced "activation followed by inhibition" (Hastings, 1970. p. 79) another unique property of physicochemical timing phenomena? Or can the clock which has been treated with drugs still sustain its normal reactions to changes in the levels of subtle geophysical factors, and thereby continue to receive and to generate information regarding the time of day, month and year?

The analyses of the effects of temperature and drugs on living clocks, several of which are discussed in later chapters, may very well help us decide whether the cellular timepieces under study are fully autonomous or are dependent upon exogenous signals. Although the nature of biological clocks is most often thought of in terms of either endogenous or exogenous, the possibility that both autonomous and environmentally modulated cellular clocks exist should be stressed. Biologists would certainly not be shocked to find timing redundancies in organisms and timing differences among organisms. Variations superimposed upon fundamental likenesses – anatomical, physiological and behavioral – are the rule in the living world of this earth.

Variations among living clocks are even more conceivable when one looks at the tremendous spectrum of types of organisms in which timing reactions have been found. Algae, protozoans, the plants and animals of most phyla and habitats are represented in our list of rhythmic species. Most of us are perfectly willing to suggest the ubiquity of cellular clocks. But are they all the same? The hands of the clocks and their mediating pathways vary tremendously. Cycles of rates of cell division, gamete production, hatching, oxygen-consumption, biosynthesis and bioelectric changes exist in many organisms. Other forms show rhythms of locomotion, movements of their parts or changes in pigmentation. A great number of these periodic changes are clearly overt and can be measured or seen obviously day after day. The circadian cycle of hatching of *Drosophila*, the fruit fly, and the tidal cycle of running of *Uca*, the fiddler crab, are overt ones.

Fewer organismic cycles are statistical ones, discernible only after the averaging of data of many hours, days or months. Mean solar-day cycles of the oxygen-consumption of potato tubers, based on data recorded for every hour of a year, have always shown three peaks – one at 7:00 AM, one at noon and the third at 6:00 PM (Brown, 1969). The tendency of mud snails to turn varies during solar and lunar periods (Chapter 6). Based on hourly observations made through a three-month period, the average circadian cycle of snails' turning had a maximum at noon with minima at 5:00 AM and 7:00 PM. Some organismic cycles may be direct reflections of the ticking of the cellular clocks. Examples are changes in the levels of respiration or the production of enzymes. Other rhythms must be mediated by chemical or nervous activities standing between the clock and its hands. Color change and locomotory rhythms are examples of these. We students of biochronometry have started to operate as comparative biologists. That approach should be continued and intensified.

The comparing and contrasting of rhythmic phenomena should not be limited to an interspecific level. Differences among cycles of individuals of one species; changes in rhythms during ontogeny; variations in them from day to day and month to month in mature organisms all should be looked into systematically. Aschoff (1960 and 1965) emphasized individual variations in frequencies under free-running conditions, and corresponding variations among ratios of animals' activity and rest. Commonly, one also finds that the rhythms of individuals of the same species exhibit different amplitudes. The amplitude of a cycle is the difference between its maximal and minimal levels. In one organism, amplitudes and average levels of a rhythm may vary "spontaneously" or after experimental treatment. The clock of the fiddler crab is not slowed down by some low temperatures, but the amplitude of its circadian cycle of color change is affected by the cold. Phasing, the temporal relationship between a specific organismic event, *e.g.*, the onset of running of an animal, and a specific time, *e.g.*, 6:00 PM, may also vary, and can be manipulated in diverse ways.

This flexibility of phasing, another major characteristic of persistent rhythms, assures the fitting of organisms' cycles to rhythms of their immediate worlds in manners adaptive to the organisms and exciting to students of biochronometry. Flying squirrels which lived under natural daylight conditions began their running about the time of sunset which varies through the year. Under laboratory conditions, that onset of activity could be set to many points in the solar period by decreasing the light intensity to which the squirrels were exposed (DeCoursey, 1960). Systematic investigations of phasing and phase-shifting are elucidating properties of those phenomena, and are teaching us much about the synchronization of events of organismic and environmental cycles by time cues, Zeitgebers (literally — time-givers) or entraining agents.

If, while in its naturally varying physical environment, an organism's cycle were to run at a frequency different from that of the solar cycle, as many circadian cycles do under constant conditions, and could not be entrained to events of the 24-hour day, its possession might become a greater liability than asset. This situation is not probable, however, for as flying squirrels and many other organisms have shown us, the phases of persistent rhythms can be set at different points in terms of actual time by particular changes or perturbations.

A cockroach can be caused to run at its peak level at most any hour by exposing it to a light to dark change at an appropriate time (Harker, 1964). The phases of the insect's rhythm will then maintain their approximate relationships with real time even under constant conditions until new Zeitgebers are presented. In its natural habitat, the roach will probably run maximally shortly after sunset even though the solar time of sunset varies through the year. If for any reason, the insect does not experience light to dark changes for a period of days, it can still be expected to be most active during early nighttime. Its cellular clock continues to direct its periodic changes in levels of its activity — approximately in time with those of its surroundings. Many living clocks, whether they be purely autonomous, environmentally cued or both, are not just luxuries. They are necessities for success in a varying world.

Let us realize that adherents of both views of the nature of the internal clock find phase-shifting of persistent rhythms explicable. The endogenous hypothesis does not assert that living systems are insensitive to changes in environmental factors — obvious or subtle ones. Proponents of this theory do claim, however, that the clock runs independent of time cues from such factors, as it does in a free-run. But when the organism is in its natural environment, its cellular clock acts with changes in its surroundings — resulting in the organismic rhythm's being beautifully in time with the physical world.

On the other hand, those who support the exogenous hypothesis do not believe that the hands of the clock are locked to particular changes in the geophysical world. The cellular clock, itself, is so locked, and is therefore modulated by these regular changes. In addition, the hands or indicators of the clock can also be affected by environmental variations. Phases of its cycles can be shifted relative to states of the internal clock, and thus to real time, by a variety of perturbations in organisms' surroundings. Several discussions of phase-shifting are included in following chapters where its great value to specific animals will be stressed.

Not all changes nor changes at all times are equally effective in entraining rhythms or shifting phases. Organisms possess rhythmic fluctuations in sensitivity to Zeitgebers. Light shocks of 10 minutes given to flying squirrels which are otherwise maintained in constant darkness shift the phases of their activity cycles only if presented during the animals' subjective night. Ten minutes of light during the squirrels' subjective daytime have no effects on phasing (DeCoursey, 1960). Are those variations in the reactions to perturbations properties of the cellular clock? Its hands? Its mediating pathways, or combinations of all those components?

Once more, the situation may vary from species to species. In the discussions to follow, the Zeitgebers most often considered will be changes in light intensity and ambient temperature. These are among the most obvious perturbations in organisms' natural habitats, and are the ones which have been most thoroughly studied in the laboratory. Sound, mechanical pressures and social cues are also effective phasing agents in some animals. Our information about them is unfortunately meager. All these findings which indicate flexibility superimposed on reliability of the timing of vital events add weight to the notion that living clocks and the phenomena cued by them – persistent rhythms, time-compensated orientation and photoperiodism – are adaptive to living systems on our earth, and undoubtedly have been of selective value during the billions of years of organic evolution which have taken place on it.

It is strange that Charles Darwin who observed and described persistent rhythms in both plants and animals well after the publication of his *opus magnum*, *The Origin of The Species*, did not stress the possible selective value of cyclic organismic activities in his monographs. However, one of his points regarding