

Conceptual Issues in the Ecology and Evolution of Circadian Rhythms

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ECOLOGY AND EVOLUTION: DISTINCT, BUT OVERLAPPING VIEWPOINTS

Many species of *Drosophila* eclose from their pupal cases at dawn. Does this eclosion occur in response to an environmental stimulus (i.e., a change in humidity, temperature, or light) or in response to an endogenous timing mechanism? A bird may fly in one direction for a few thousand kilometers, then turn and fly in another direction. Is the bird responding to landmarks or magnetic cues, or does a biological clock determine how long the bird flies in a particular direction during migration? These questions and others like them are important if we are to understand *how* the behavior* of organisms in their natural environment is regulated and *why* it is regulated as it is.

Ecological studies examine the proximate mechanisms underlying the interactions of individuals within their communities or ecosystems. These mechanisms are described at a very different level of biological organization than the molecular and biochemical mechanisms described in other chapters of this book, but the essence of the question is the same: *How* is a system regulated? In this case, the system consists of the organism and its environment rather than the biochemical processes

*For the sake of brevity, "behavior" will be used throughout this chapter to refer both to behavior and internal physiologic processes such as hormonal secretions, metabolism, etc.

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within a single organism or cell. When an ecologist tests hypotheses about which factors regulate the timing of eclosion of fruit flies in the genus *Drosophila* in their natural environment, he or she is studying the mechanism responsible for regulating that behavior in the context of the community to which the flies belong.

Organisms are complex and can adapt to their environments by modifying many physiologic and behavioral mechanisms other than their temporal organization. For example, *Drosophila* are hypothesized to eclose at dawn to avoid desiccation. Most shizophorans, the subfamily of insects that includes the genus *Drosophila*, are day-flying insects with excellent abilities to withstand desiccation (Cloudsley-Thompson, 1960). In contrast, flies in the genus *Drosophila* are hypothesized to have become adapted to arid environments by increasing their visual acuity to low light intensities, which enabled them to confine their activity to darker and more humid times of day (Cloudsley-Thompson, 1960). Evolutionary biologists would ask why this pattern evolved: What were the pressures that resulted in *Drosophila* exhibiting this crepuscular (i.e., active at dawn and dusk) pattern? An evolutionary biologist would also ask whether the crepuscular pattern preceded or followed the invasion of *Drosophila* into an arid habitat to determine whether the crepuscular pattern was an adaptation to life in the arid environment or a preexisting condition that allowed *Drosophila* to invade an open niche. By asking evolutionary questions, we may better understand the fundamental principles which determine the temporal organization of behavior and the principles which determine the mechanisms controlling that behavior. Conversely, studies of circadian rhythms may provide a useful example for the study of complex organisms. Circadian rhythms and the evolution of the temporal organization of behavior can be used to study the fundamental concept of the coevolution of physiology, morphology, and behavior.

Despite distinct intellectual traditions, the fields of ecology and evolutionary biology merge at a broad interface. If an ecologist wishes to test the hypothesis that a particular behavior is an adaptation, she or he needs to understand the phylogeny of the behavior to know whether or not it has been modified from an ancestral condition to meet the demands of the current environment. If an evolutionary biologist wishes to test the hypothesis that the control of a particular behavior by a circadian clock is adaptive, he or she needs to understand the ecology of the organism because it is the interaction of the organism with its environment that will determine the success of the organism and its offspring.

Circadian rhythms have been observed in virtually every living organism, prokaryotic as well as eukaryotic (Crosthwaite, Dunlap, & Loros, 1997; Kay, 1997; Kondo, Golden, Ishiura, & Johnson, 1994). It is beyond the scope of this chapter to provide a comprehensive review of the diverse behaviors and physiologic processes known to be under the control of endogenous circadian pacemakers; in general, circadian controlled behaviors are known to include, but are not limited to, sexual behavior, the sleep-wake cycle, nest building, torpor, feeding, migration (in aquatic and terrestrial environments), luminescence, and several gated developmental rhythms (Daan, 1981; DeCoursey *et al.*, submitted; Enright, 1970).

This chapter summarizes and integrates concepts of ecology and evolutionary biology with observations about circadian rhythms. The goal of this summary and integration is to encourage animal behaviorists, ecologists, evolutionary biologists, and molecular biologists to work hand in hand to elucidate the fundamental biological principles underlying the diversity of both the structure of circadian clocks and the function of circadian rhythms.

The things an organism must do in order to survive and reproduce in nature are find food, find a mate (if it is a sexually reproducing organism), find habitats with optimal conditions for survival (e.g., temperature, humidity, light intensity, pH, oxygen availability, etc.), and avoid predators. The niche occupied by an animal in its community is defined by the interactions of an organism with the biological and physical factors which contribute to its survival and subsequent reproduction. Given the earth's daily rotation, these biological and physical factors are not uniformly distributed throughout the day-night cycle. Thus, the temporal structure of an organism's environment is an equally important component of its niche as the spatial, chemical, energetic, and social components. This temporal structure in the environment contributes to the segregation of the activities of individual animals to specific times of day. The temporal structure of the environment also contributes to the temporal segregation of the activities of entire species which may contribute to the diversity of species that inhabit a community (Schoener, 1970, 1974). Investigations of the mechanisms, including circadian rhythms, by which organisms respond to the temporal structure of their environment should be central to studies of ecology. The question of interest to circadian biologists and ecologists is whether the mechanisms regulating this temporal order (1) rely on a direct stimulus-response relationship between the animal and its environment, (2) rely on an internal clock, or (3) represent the interaction between the external environment and an internal clock.

INFLUENCE ON THE BEHAVIOR OF INDIVIDUALS

One objective of research into the temporal organization of behavior should be to describe correctly the relative contributions of external stimuli and endogenous rhythms when testing hypotheses regarding the organization of behavior under natural conditions (Enright, 1970; Zucker, 1983). A fundamental assumption in ecology and evolution is that individuals which maximize their energy consumption while minimizing their exposure to predation or other stressors are most likely to survive and reproduce in a given environment (Bennett, 1987; Horton & Rowsemit, 1992). The degree to which external and internal factors regulate the daily temporal program may be determined by the level of correlation (*predictability*) in the occurrence of environmental events from one cycle to the next. Through effects on survival and reproduction, behaviors that coincide with events which are highly predictable from cycle to cycle may be more tightly controlled by an internal program than those associated with environmental conditions having lower levels of predictability (Pittendrigh & Daan, 1976b; Rapp, 1987).

The ability to anticipate a coming change in the environment is provided by a circadian clock. A timing mechanism built on the foundation of an oscillator with a stable cycle length (i.e., *free-running period*, τ) permits behaviors that require the measurement of elapsed time (e.g., time-compensated sun orientation, time-sense [Zeitgedachtnis]; Enright, 1970; Pittendrigh & Daan, 1976b). The presence of an oscillator also provides organisms with the ability to recognize local time. This recognition of local time results in the synchronization of activities with the environmental cycle producing the condition known as *entrainment* (Enright, 1970; Mrosovsky, 1996; Pittendrigh & Daan, 1976b). Entrainment is characterized by a stable relationship (*phase angle* ψ) between specific environmental events (e.g., dawn or

dusk) and specific behaviors (e.g., the onset of activity). Desert-dwelling kangaroo rats (genus *Dipodomys*) that emerge from their underground burrows within ½ hour of sunset each day are exhibiting entrainment resulting from exposure to the light-dark cycle and, perhaps, social interactions (Kenagy, 1973; Mrosovsky, 1996).

For behaviors known to be under circadian control, the oscillatory model of circadian rhythms can function as a starting point, or null hypothesis, for predicting patterns of behavior under a variety of environmental conditions. For example, two characteristics of circadian rhythms are that τ is not necessarily equal to 24 hours (i.e., is "circa" = about, "dian" = a day) and that τ is quite stable. The functional significance of these characteristics becomes apparent when examined from the perspective of the conservation of a stable phase angle (e.g., maintaining a constant onset of activity relative to sunset). A critical analysis of laboratory data from nocturnal rodents and from mathematical models of oscillators reveals that the deviation of τ from 24 hours and its low variability contribute to the conservation of phase angle (Pittendrigh & Daan, 1976b). These characteristics predict that organisms should maintain a stable phase angle under seasonal changes in photoperiod (or day length) (Pittendrigh & Daan, 1976b). The observation of deviation from these predictions in organisms under natural or controlled laboratory conditions would indicate that other factors, either in the external environment or within the organism, are modifying the regulation of the behavior by the circadian system.

There are several examples from nature in which the phase angle is not conserved for activities known to be under circadian control (Daan, 1981; Kenagy, 1976). Kangaroo rats of the genus *Dipodomys* show seasonal changes in ψ (Kenagy, 1976). There is no correlated seasonal change in τ that could account for this change in ψ . The most likely explanation is that the kangaroo rats are responding to the seasonal change in the length of twilight (Kenagy, 1976, 1978). Other examples can be found in the arvicoline rodents (e.g., voles, lemmings, muskrat; Wilson & Reeder, 1993). These rodents frequently shift their activity from a primarily nocturnal pattern in the summer to a crepuscular or diurnal pattern in the winter (Erkinaro, 1969; Hoogenboom, Daan, Dallinga, & Schoenmakers, 1984; Rowsemitt *et al.*, 1982). One proximate mechanism for this shift appears to be a seasonal change in gonadal steroids; high testosterone facilitates nocturnal activity (Rowsemitt, 1986). However, temperature is also a contributing factor; crepuscular voles switch back to nocturnal patterns of activity during the winter if temperatures become unseasonably warm (Claypool, 1984).

These observations indicate that while a circadian clock can regulate temporal patterns of behavior in individuals, animals are not slaves to the physiology of their circadian clocks. As with all models in biology, models of behavior based on circadian principles provide a hypothesis. When behavior is observed to deviate from the hypothesis, new investigations lead us to a better understanding of the actual mechanisms responsible for the regulation of behavior. These new investigations enhance our understanding of both the behavioral ecology of animals and the mechanisms by which circadian rhythms regulate behavior.

CIRCADIAN MODELS AND THE ORGANIZATION OF COMMUNITIES

The species within a community may also partition the temporal environment to avoid or reduce competition. Schoener (1970, 1974) observed that different species of lizards of the genus *Anolis* sunned themselves on the same branches, but at different times of day. An additional example can be taken from data collected to

test hypotheses derived from the theory of optimal foraging (Kotler, Brown, & Subach, 1993). These data were not collected under conditions necessary to determine the role of an endogenous timing mechanism, but they provide an example for which it may be fruitful to ask such questions. The study examined a community of desert-dwelling, granivorous gerbils (*Gerbillus pyramidum* and *G. allenbyi*) in Israel. The larger species (*G. pyramidum*) prefers to forage in patches with high seed densities. During the course of a night, individuals of the larger species emerge first, forage until the seed densities are reduced to suboptimal levels, then retreat to their burrows. The smaller species then emerges and forages until seed densities are further reduced. If *G. pyramidum* is removed from the community, the timing of activity by *G. allenbyi* shifts to an earlier time. The investigators hypothesized that aggressive encounters between the species maintain the temporal feeding hierarchy (Kotler *et al.*, 1993). A similar pattern of temporal partitioning is seen in a community of desert-dwelling heteromyid rodents (kangaroo rats and pocket mice) in North America (Kenagy, 1973).

From a chronobiologist's point of view, one would test the following alternative hypotheses: (1) that the aggressive encounters regulate a direct stimulus-response system and (2) that the aggressive encounters function as a nonphotic stimulus to entrain the endogenous pacemakers of the various members of the community (Honrado & Mrosovsky, 1991; Mrosovsky, 1996). If an endogenous timing mechanism is involved, it may limit the range of time domains that individuals of a species could occupy if the other species in the community were removed or new ones added (Pittendrigh & Daan, 1976a, c). The implication that the formal properties of an endogenous clock may restrict the possible patterns of temporal organization even when specific environmental stimuli are added or removed is important to ecologists. Since the goal of ecology is to understand the mechanisms organizing the temporal structure of individual behavior or interactions within communities and ecosystems, the appropriate roles must be ascribed to external stimuli and endogenous clocks.

EVOLUTIONARY BIOLOGY AND THE STUDY OF CIRCADIAN RHYTHMS

Circadian rhythms are striking in the ubiquity with which they occur, in the similarity of the formal properties of rhythms among diverse groups of organisms, and in the apparent similarities in the molecular structure of pacemaker molecules. These similarities lead to the hypothesis that the cellular pacemakers upon which circadian clocks are built arose early in the history of life on earth and that common elements may be shared among all organisms today (Crosthwaite *et al.*, 1997; Kay, 1997; Kondo *et al.*, 1994). Circadian rhythms are also striking because of the diversity of anatomic structures and biochemical processes that make up complex clock systems (i.e., the integrated system consisting of input mechanisms, pacemaker(s), and output mechanisms) (Menaker & Tosini, 1996; Page, 1989; Roenneberg, 1994). This observation leads to the hypothesis that complex clock systems built on the foundation of a circadian pacemaker have evolved repeatedly (i.e., represent *convergent* evolution) in diverse groups of organisms. Finally, circadian rhythms are striking because of the intricate temporal correlations between the expression of behavior and the occurrence of environmental events in specific habitats (Daan, 1981; Enright, 1970; DeCoursey, 1990). This observation leads to the hypothesis that the expression of rhythmic behavior is easily modified by natural selection as organisms

adapt to their specific environments. All three of these evolutionary hypotheses are amenable to testing using comparative methods.

"Evolution" simply means change over time. Starting with the principle of common ancestry, Darwin proposed that there should be a degree of similarity among closely related organisms (Darwin, 1859). However, Darwin also recognized that organisms change over time and that at least some of this change was related to local environmental conditions. Therefore, one must consider both phylogenetic and environmental components if one is to develop a complete understanding of the ubiquity, similarity, and diversity of circadian clocks. While it is not possible to reconstruct the environments in which organisms evolved, we do know what environments organisms inhabit today. The patterns of existing diversity can inform us about the pressures required to maintain circadian pacemakers and the clocks built on their foundation. The information about current conditions can be used to infer what happened in the past (Harvey & Pagel, 1991, p. 40). As with all scientific inquiry, one can never really prove that a hypothesis is correct; however, with careful analysis, it is possible to reject certain alternatives. The persistent testing of hypotheses using comparative methods that consider both phylogenetic and environmental contributions to diversity can be used to build a testable theory of the fundamental organizational principles that underlie the evolution of circadian pacemakers, complex circadian clocks, and the daily temporal expression of behavior.

COMPARATIVE BIOLOGY AND TESTS OF HYPOTHESES OF COMMON ORIGIN AND CONVERGENCE

Two of the hypotheses described above (i.e., regarding the origin of pacemakers and the evolution of complex clock systems) describe questions directed at different levels of analysis of circadian rhythms, but the underlying problem is the same: What is the evolutionary origin of an observed characteristic? The hypotheses that characteristics are derived from a common ancestor or evolved independently by convergent evolution are alternative hypotheses that can be tested at many different levels of analysis.

Evolutionary biologists test alternative hypotheses about the origins of characteristics by looking for patterns of similarity. The theoretical foundation known as *phylogenetic systematics*, which is commonly used by evolutionary biologists to estimate phylogenies, was provided by Hennig (1950, 1966). In principle, the construction of a phylogeny is based on the assumption that there are three types of homologies among traits (Brooks & McLennan, 1991, pp. 24–68; Harvey & Pagel, 1991, pp. 50–77). Shared *general homologies* are common to all members of a taxonomic group (e.g., an internal skeleton is a shared general homology of all vertebrates). Shared *special homologies* are common to specific subgroups within the larger taxonomic group (e.g., fur is a shared special homology of mammals). Finally, *unique homologies* identify individual taxa within groups (e.g., the modification of the forelimbs into wings is a unique character of the Order Chiroptera [bats]).

The same analytical procedures can be used to construct phylogenies of single molecular components of circadian clocks or of entire clock systems. The investigator first needs to define the aspect of circadian clocks under examination (i.e., photoreceptor, input system, pacemaker, output system, or the complete structure of the integrated clock system). He or she then develops a data matrix containing information on the characters (traits) which comprise the system under investigation. The matrix needs to include information about whether or not the characters

differ from the presumed ancestral state. The ancestral condition is determined by comparison with an *outgroup*, a group that is very distantly related to the group being examined. The data matrix is then analyzed using computer software specifically designed to generate phylogenetic relationships and establish the statistical significance of the phylogenetic relationships (see Hillis, Moritz, & Mable, 1996; Swofford, Olsen, Wadell, & Hillis, 1996; Wiley, 1981, for details of these procedures). Because the assumptions and mathematical weighting of variables differ among algorithms, evolutionary biologists frequently examine their data using multiple algorithms to look for instabilities in the branching patterns of the phylogenies. Instabilities indicate uncertainties in the predicted phylogenetic relationships.

Tests of hypotheses about the origin of circadian pacemakers must await the accumulation of additional data about the structure and function of the molecules that make up the pacemakers of diverse organisms. However, the current model of circadian pacemakers proposes a cellular feedback loop involving the regulation of gene transcription through the interactions of several different genes and their protein products (Reppert & Weaver, 1997). Examples of these interactions are seen in *Neurospora* (e.g., *fig* and *wc-2*) and in *Drosophila* (e.g., *per* and *tim*) (Crosthwaite *et al.*, 1997; Gekakis *et al.*, 1995; Sehgal *et al.*, 1995). An emerging theme in the molecular biology of circadian pacemakers is the presence of a PAS domain, required for protein dimerization, in at least some pacemaker molecules from *Neurospora*, to *Drosophila*, to humans (Albrecht, Sun, Eichele, & Lee, 1997; Crosthwaite *et al.*, 1997; Huang, Edery, & Rosbash, 1993; Kay, 1997; King *et al.*, 1997; Shearman, Zylka, Weaver, Kolakowski, & Reppert, 1997; Shigeyoshi *et al.*, 1997). Phylogenetic procedures have been employed to examine the phylogenetic relationships of PAS-domain-containing molecules (e.g., those from *a.p.per*, *d.m.per*, *hper1*, *mper1*, *hper2*, *mper2*, *hper3*, and *mClock*; Shearman *et al.*, 1997) and to examine variation in the structure of *per* molecules in different populations of *Drosophila* (e.g., Rosato, Peixoto, Barbujani, Costa, & Kyriacou, 1994). To test the hypothesis that extant pacemakers are derived from a common ancestor will require in-depth assessment of the molecular structure of the different genes and proteins which make up the pacemakers of multiple species each of prokaryotes and eukaryotes.

The function of putative pacemaker molecules should also be subjected to analysis (Sassone-Corsi, 1996). Recent data indicate that structurally related proteins may have different functions in the pacemakers of different species. For example, the cellular patterns of expression of *per* are very different in the silkworm (*Antheria pernyi*) and in fruitflies (*Drosophila* spp.) (Sassone-Corsi, 1996; Sauman & Reppert, 1996). The different patterns of expression in *A. pernyi* and *Drosophila* provide tantalizing data to suggest an alternative hypothesis for the origin of pacemakers, that certain types of molecules are easily recruited to contribute to a pacemaker mechanism, but that the evolution of the pacemaker itself may have occurred repeatedly by convergent evolution. Differences in expression also are observed among *per*-like proteins in *Drosophila* and mice which suggest significant differences in function (Albrecht *et al.*, 1997; Shearman *et al.*, 1997; Shigeyoshi *et al.*, 1997). The current fountain of molecular data flowing from laboratories should, in time, provide sufficient data on the structure and function of molecules that comprise circadian pacemakers in a variety of organisms to permit testing of hypotheses about the origin of circadian pacemakers.

The same procedures can be employed to analyze the evolution of other components of circadian clocks (i.e., photoreceptive pigments, *N*-acetyltransferase molecules) or the evolution of complete clock systems (Klein *et al.*, 1997; Menaker &

Tosini, 1996; Soni & Foster, 1997). For example, within the vertebrates, mammals appear to be unique in the extent to which pacemaker function is centralized in a single brain region, the suprachiasmatic nucleus. It is hypothesized that this represents the passage of mammals through an evolutionary bottleneck as small nocturnal organisms during the Cretaceous period (Menaker & Tosini, 1996). Several other nocturnal species of vertebrates also show an increased reliance on the hypothalamic pacemaker and a reduction in importance of the retinas or pineal/parietal complex as pacemakers (e.g., owls, geckos, hagfish) (Menaker & Tosini, 1996). Comparison of closely related nocturnal and diurnal species from each of these groups would permit testing of the hypothesis that the reliance on the hypothalamic pacemaker evolved repeatedly by convergence in nocturnal animals.

COMPARATIVE BIOLOGY AND TESTS OF ADAPTATION OF CIRCADIAN RHYTHMS

The repeated appearance of similar characteristics in diverse groups of organisms is often assumed to indicate that the organisms are adapting to a common environmental pressure. For example, it is hypothesized that differences in sensitivity to light among desert- and forest-dwelling rodents may represent adaptation to the different light environments of these habitats (DeCoursey, 1990). The rodent species compared in this study (forest dwellers: *Glaucomys volans* [flying squirrel] and *Peromyscus leucopus* [white-footed mouse]; desert dwellers: *Dipodomys merriami* [kangaroo rat] and *Mesocricetus auratus* [golden hamster]) are phylogenetically distinct. These ecological data provide the basis for the hypothesis of adaptation. To test the hypothesis that a characteristic is adaptive, it is necessary to demonstrate that (1) variability in the characteristic exists, (2) the observed variation has a genetic basis, (3) variability in the trait leads to nonrandom differences in fitness of the individuals in a population, and (4) the observed trait evolved in response to the hypothesized environmental pressure (Brooks & McLennan, 1991; DeCoursey *et al.*, submitted; Endler, 1986; Gould & Lewontin, 1979; Harvey & Pagel, 1991). These criteria have been met in some studies of circadian rhythms, but data are often insufficient to permit an actual test of the hypothesis.

The fundamental observation that circadian rhythms are an innate, genetically based characteristic of organisms is well established (Aschoff, 1960; Bünning, 1960; Takahashi, 1995). Variability in the length of the free-running period of the circadian pacemaker is frequently observed both within and between individuals. This variability is, in fact, a defining characteristic of the circadian nature of a rhythm (Aschoff, 1960). There is evidence for genetic differences in the responsiveness of the circadian system to light (Pohl, 1983; Pittendrigh, Kyner, & Takamura, 1991; Possidente & Hegmann, 1982; Shimomura *et al.*, 1998). There is evidence of a latitudinal cline in the ability of *Drosophila auria* to phase shift in response to light pulses (Pittendrigh *et al.*, 1991). Variation is also observed in the structure and function of individual components of clock systems of organisms that inhabit different niches. In one of the most thoroughly studied systems, variation in the length of the threonine-glycine repeat of the *per* gene in *Drosophila melanogaster* and *D. simulans* has been shown to occur on a latitudinal gradient. This variation is associated with the stability of the free-running period under different temperatures (i.e., *temperature compensation*) (Costa, Peixoto, Thackeray, Dalglish, & Kyriacou, 1991; Costa, Peixoto, Barbuji, & Kyriacou, 1992; Rosato *et al.*, 1994; Sawyer *et al.*, 1997).

The selective advantage of circadian rhythms is hypothesized to result from increased *fitness* (i.e., increased reproductive success) that individuals capable of

measuring the lapse of time or recognizing local time may accrue (Bünning, 1971; Daan, 1981; Daan & Aschoff, 1982; DeCoursey *et al.*, submitted; Enright, 1970; Pittendrigh & Daan, 1976b). When evaluating the significance of changes in individual clock components, it is important to remember that it is the overt behavior of an organism that interacts with the environment. It is the whole organism that must survive and reproduce, therefore the impact of genetic variation in any single clock component will be determined by its effect on the survival and reproductive fitness of the individual. Therefore, alteration of clock components that result in significant changes in patterns of entrainment are most likely to be subjected to natural selection. Mutations that do not result in a significant change in the pattern of entrainment may accumulate in the genome as neutral mutations.

There are few experimental studies that directly test the hypothesis that the presence of a clock, or a clock exhibiting specific characteristics, actually increases fitness. Early studies suggested that exposing organisms (e.g., tomatoes, fruitflies, and blowflies) to light-dark cycles with periods significantly different from 24 hours was harmful (Hillman, 1956; Pittendrigh & Minis, 1972; Saint Paul & Aschoff, 1978). Recent work using a diurnal mammal, the antelope ground squirrel (*Ammospermophilus leucurus*), demonstrates that animals bearing suprachiasmatic lesions behave normally in the field and laboratory except in one aspect: the animals become arrhythmic (DeCoursey, Krulas, Mele, & Holley, 1997). This arrhythmia results in animals being active at night as well as during the daytime. An example of how this arrhythmia may decrease fitness is the fact that twice as many of the lesioned animals than control animals (60% versus 29%) were killed by the nighttime invasion of a feral cat onto the study site. Field observations of naturally occurring populations of guillemots (*Uria lomvia*) suggest that young birds that fledge from their nests late in the afternoon, in synchrony with the rest of the population, have a greater survival rate than those that do not (Daan & Tinbergen, 1980). While it is an example of a circatidal rhythm rather than a circadian rhythm, marine iguanas that time their movement to the beach to closely match the time of low tide have higher long-term survival than those that do not (Wikelski & Hau, 1995).

Finally, to demonstrate that a character is an adaptation that has arisen in response to a specific environmental pressure, rather than a preexisting permissive condition or a characteristic correlated with some other physiologic change, one must compare ancestral and derived traits (Gould & Lewontin, 1979; Harvey & Pagel, 1991, pp. 13-34). One method currently being used to test the adaptive significance of circadian rhythms is to look for the loss of rhythms under conditions where they appear to present no clear advantage. A number of species have removed themselves from exposure to environmental periodicity by becoming fossorial, inhabiting caves or benthic habitats (Goldman, Goldman, Riccio, & Terkel, 1997; Jegla & Poulson, 1968; Kenagy & Vleck, 1982; Lovegrove & Muir, 1996; Poulson and White, 1969). Kenagy and Vleck (1982) proposed four conditions that could result in relatively uniform day-to-night levels of resting metabolic activity in mammals: (1) continuously fossorial habit, (2) consumption of bulky, low-quality food requiring frequent foraging, (3) small body size, near the lower limit of mammalian body size, and (4) the ability to hibernate.

Patterns of locomotor activity, body temperature, and metabolic activity have been determined for several different species of fossorial mammals from different orders and suborders; the degree to which they express circadian rhythms is highly variable within and between species. Locomotor activity of blind mole rats (*Spalax ehrenbergi*, Order Rodentia, Suborder Sciurognathi, Family Muridae) and the unre-

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