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Introduction

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One contribution to a Special Feature 'Animal clocks: when science meets nature'.

Animal clocks: when science meets nature

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Daily rhythms of physiology and behaviour are governed by an endogenous timekeeping mechanism (a circadian 'clock'), with the alternation of environmental light and darkness synchronizing (entraining) these rhythms to the natural day–night cycle. Our knowledge of the circadian system of animals at the molecular, cellular, tissue and organismal levels is remarkable, and we are beginning to understand how each of these levels contributes to the emergent properties and increased complexity of the system as a whole. For the most part, these analyses have been carried out using model organisms in standard laboratory housing, but to begin to understand the adaptive significance of the clock, we must expand our scope to study diverse animal species from different taxonomic groups, showing diverse activity patterns, in their natural environments. The seven papers in this Special Feature of *Proceedings of the Royal Society B* take on this challenge, reviewing the influences of moonlight, latitudinal clines, evolutionary history, social interactions, specialized temporal niches, annual variation and recently appreciated post-transcriptional molecular mechanisms. The papers emphasize that the complexity and diversity of the natural world represent a powerful experimental resource.

1. Introduction

Since the dawn of life, most organisms have had to adapt to environmental cycles of light and darkness, and restrict many of their biological activities to specific times of day and night. Central to this adaptation is the evolution of an endogenous, self-sustained, 24 h (circadian) timekeeping mechanism ('clock') that orchestrates body rhythms for concerted action and synchronizes (entrains) them to the local time of day [1]. In the last few decades, there have been spectacular advances in our understanding of this clock mechanism. It is a layered system with emergent properties at several levels of organization, including regulatory molecules, cells, circuits and tissues. It is believed that genes at the clock's core function as autoregulatory feedback loops within individual cells, with oscillating levels of nuclear proteins negatively regulating the transcription of their own mRNAs. Groups of autonomous single-cell oscillators are coupled together to form discrete pacemakers that generate coherent outputs for expression of overt rhythms with a diverse range of tissue-, species- and developmentally specific waveforms and phases. The pacemaker works as a clock because its endogenous period is adjusted to the external 24 h period, primarily by light-induced phase shifts that reset the pacemaker's oscillation. Variations in photic sensitivity, in concert with changes in the pacemaker's endogenous period and amplitude, can dramatically affect the phase of entrainment of clock-controlled events. It is thought that the selective advantages of a clock are for optimizing the economy of biological systems, enabling plasticity of responses to an altered environment, and allowing for predictive, rather than purely reactive, homeostatic control.

By necessity and design, elucidation of circadian mechanisms has mostly relied on using model organisms that are amenable to genetic manipulation and standard laboratory housing. But the power of this approach comes with certain limitations, especially when asking questions about the ecological and evolutionary implications of these mechanisms. To begin to understand the functional significance of the clock and the selection pressures at the evolutionary scale that have shaped patterns of animal rhythmicity, we must expand our

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scope to study diverse animal species from different taxonomic groups showing diverse activity patterns and in their natural environments, with, for example, changing temperature and humidity, limited resources, cooperation or competition from conspecifics and the presence of predators.

The shortcomings of an over-reliance on model organisms are already well known, as 'studying only a few organisms limits science to the answers that those organisms can provide' (p. 31 of [2]). Indeed, important aspects of the physiology and behaviour of wild animals may not be mirrored by their inbred models. Consider the example of melatonin, an indolamine hormone produced by the pineal gland and secreted during the night. It is involved in circadian and annual (seasonal) rhythms, and has a critical role in regulating seasonal reproduction, with an anti-gonadal effect in long-day breeders and a pro-gonadal effect in short-day breeders. Surprisingly, most inbred strains of laboratory mice are melatonin-deficient [3,4]; in the case of C57BL/6J mice, for example, this is due to mutations in the genes that encode enzymes in the melatonin biosynthetic pathway [5,6]. Given the inhibitory effects of melatonin on murine testis development, it is suspected that reproductive success in the laboratory has been the selective pressure favouring melatonin deficiency in commercial breeding colonies. Such success in the cage clearly does not translate to success in the real world, where the effects of changing day lengths on melatonin production help to mark the breeding season most conducive to offspring survival.

The natural environment is much more complex than the laboratory environment in which studies of model organisms are typically performed [7], so mechanistic insights gleaned from the laboratory may not translate simply to the wild. The typical circadian laboratory investigates clock period and phase under rigid (usually 12 L : 12 D) cycles, as well as constant lighting conditions (continuous darkness or light) to eliminate possible 'masking' influences of light, which override or circumvent the clock mechanism. Obviously, these conditions differ greatly from what is experienced by individuals in their natural habitat. Nature provides a much richer and more challenging cycling environment, including factors that are abiotic (temperature, humidity, radiation, and light of variable intensity and spectral composition at dawn and dusk) as well as biotic (interspecific and conspecific interactions, food availability). All these influences may dramatically affect adaptive physiology and behaviour, and indeed there are examples that show activity patterns for the same species, or even the same individual, differing considerably between the laboratory and natural environment (reviewed in [7,8]). Masking responses, often viewed pejoratively in the laboratory, have clear adaptive significance under natural or semi-natural conditions in the field, both in nocturnal and diurnal mammals [8]. What we have learned about clocks and rhythms in model organisms in the laboratory can now set the stage for a new generation of comparative studies that exploit natural variation and complex habitats.

Diurnality is an interesting example that illustrates the value of expanding research along less conventional lines. Research on animal rhythms has focused primarily on nocturnal rodents, although studies of diurnal species are on the rise. This work has revealed that some very fundamental features of the circadian system are the same in apparently diurnal and nocturnal animals, including the molecular oscillatory machinery and the mechanisms responsible for pacemaker entrainment by light (reviewed in [9]). Comparative studies of rhythms in diurnal and nocturnal species suggest that the fundamental differences between them emerge downstream from the master pacemaker in the suprachiasmatic nucleus (SCN) of the hypothalamus [10], specifically in the mechanisms that couple the SCN to overt activity and related functions, including eating, drinking, copulating, giving birth and nursing young, as well as a host of other activities [11]. These differences often do not reflect a simple 180° reversal in phase, as their waveforms may vary considerably as well [12,13]. Another important principle that has come from recent studies of diurnal rodents involves plasticity. Under certain conditions, activity patterns of diurnal animals may become quite nocturnal, and vice versa. Such plasticity (likely to be due to altered coupling of the rhythms of subsidiary brain oscillators to that of the SCN) may help animals accommodate changes in the natural environment that make night-time activity more suitable. Of note, some animals appear to be fundamentally optimally adapted for daytime activity, not night-time activity, and vice versa [14]. Sensory systems best suited for daytime activity are different from those for night-time activity; sleep is fragmented when diurnal animals recover from an active night; and in at least one species, the diurnal Nile grass rat (*Arvicanthis niloticus*), it appears that some brain oscillators and behavioural patterns are not always congruent [15]. As chronobiologists seek to translate their findings in animals to (diurnal) humans and the pathophysiology of disease, a deeper understanding of diurnality in complex settings will be of paramount importance.

The seven papers in this Special Feature of *Proceedings of the Royal Society B* take on the challenge of investigating clocks and rhythms in nature, inspired by an interdisciplinary workshop held in Ein Gedi, Israel in 2012, entitled 'The Diversity, Evolution and Mechanisms Controlling Activity Patterns'. Such patterns, and the mechanisms that control them, are of great significance for understanding subjects such as behavioural evolution (the selective forces leading to different patterns), behavioural ecology (the use of time as a niche axis and the role of activity patterns in shaping community structure) and behavioural neuroscience (the neural substrates regulating activity rhythms). Together, the contributions take on a range of non-traditional questions, from post-transcriptional processes within clock cells up to sociobiological interactions between individuals in a group and between species in an ecological community.

While the pre-eminent role of sunlight in regulating animal activity patterns has been universally appreciated, what about moonlight? In the first paper of this series, Kronfeld-Schor *et al*. [16] review the growing lines of evidence suggesting that lunar light significantly influences the activity patterns of both diurnal and nocturnal animals. They discuss the possible adaptive value of moonlight-modulated activity patterns and describe possible mechanisms underlying this effect. Importantly, they also address other sources of night-time light, specifically those associated with modern human activity ('light pollution'), and suggest that such increased illumination, especially in modern urban areas, may alter circadian clock function and control of downstream processes such as those related to reproduction.

The Earth's elliptical orbit and its axial tilt give rise to annual systematic variations in photoperiod and thermoperiod that depend on latitude, with extremes at the poles. Hut *et al*. [17] explore the basis and possible mechanisms for temporal adaptation to life at different latitudinal clines, including initial characterization of clock-gene polymorphisms. They point out intriguing correlations between latitude and circadian properties, including overt rhythm amplitude, phase of entrainment and free-running period, and make the compelling case that study of latitudinal variation can lead to fresh insights on the selection pressures and mechanisms that have shaped the evolution of daily and annual timekeeping.

Evolutionary history is also considered by Gerkema *et al*. [18], who revisit the 'nocturnal bottleneck' hypothesis, which states that ancestral placental mammals evolved nocturnality as a strategy to minimize interactions with the dominant and mostly day-active dinosaurs. The authors integrate evidence from multiple disciplines, including recent palaeontological findings, research on the ecology and physiology of extant vertebrate species, and molecular phylogenetic reconstructions of genes involved in light detection or protection. Their verdict is that, overall, the data are consistent with the hypothesis, which continues to provide the most plausible explanation for observed activity patterns in mammals.

Outside the laboratory, animals interact with other individuals, in some cases synchronizing their daily behaviour with that of conspecifics. Bloch *et al*. [19] review studies with three model systems for mutual oscillator synchronization, from colonies (honeybee societies) and organisms (social interactions in fruitflies) to cells (intercellular coupling in the SCN). They found in all these levels of organization that interactions among oscillators can lead to emergent group-level circadian phenotypes that cannot be predicted simply based on the properties of any single oscillator; they highlight the need to better understand the mechanisms underlying these complex interactions at multiple levels of organization.

Despite the view that normal circadian rhythms are crucial for animal health and survival, some animals show extended periods of activity around the clock with weak or no circadian rhythms—and no apparent ill effects. Bloch *et al*. [20] suggest that activity around the clock with no overt circadian rhythmicity is more common than is currently accepted and may be functionally adaptive for animals living in constant environments or with specific life-history traits such as long migrations or advanced sociality. They further review studies on the possible underlying mechanisms and hypothesize that the complexity of the circadian system enables activity around the clock, while simultaneously keeping vital processes under appropriate circadian regulation.

Helm *et al*. [21] focus on phenology, the annual timing of recurring biological processes, and the interface between environmental cues and mechanistically diverse internal oscillators, ranging from timers that measure intervals of several months to endogenous circannual clocks. The authors suggest that mismatches between these calendar mechanisms and recent environmental changes brought about by global warming and urbanization can have severe ecological implications. They propose that viewing our own species as a 'seasonal animal' may inspire novel approaches for addressing medical and psychological disorders in humans.

Finally, Bartok *et al*. [22] take on the problem of how circadian clocks are both stable (expressing a relatively constant circadian period over a broad range of ambient temperatures) and labile (with rapid and predictable resetting in response to timed photic and thermal stimuli). The authors focus on the molecular clock and on the role of two key post-transcriptional processes—alternative splicing of pre-mRNAs and microRNA modulation of gene expression—that regulate mRNA stability and translational efficiency. They review recent studies in fungi, plants, fruitflies and mice suggesting that these mechanisms are critical for mediating the rapid responses of circadian clocks to environmental cues.

Of course, this collection raises more questions than it answers about the opportunities and challenges confronted by animals living on our rotating planet—questions that demand an array of specialized behavioural and physiological responses. But for researchers seeking to understand the adaptive significance of animal clocks, the foremost experimental resource is surely the complexity and diversity of the natural world.

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