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Review Article

Biological clocks and life-histories

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Abstract

Life-histories are all about the timing of key events related to reproduction in organisms, and most organisms possess chronometers in the form of circadian clocks. Thus, it is reasonable to ask whether circadian clocks play a role in determining the timing of important life-history events. A better appreciation of the ubiquitous effects of circadian clocks on various behavioural and physiological traits also has implications for the design and interpretation of experiments in life-history evolution. In this article, studies—mostly using *Drosophila*—that explore the links between circadian organisation and life-history related traits are briefly reviewed, and their implications for life-history evolution studies are discussed.

Keywords: time, adaptation, circadian organisation, development, lifespan, life-history evolution, *Drosophila*.

Introduction

Evolutionary biology and chronobiology have remained largely separate fields over the years, with few points of contact between them (Sharma and Joshi 2002). On the one hand, a small number of studies in chronobiology have attempted to address the adaptive significance of circadian organization, as well as its evolutionary fine-tuning (Sharma 2003a). For example, studies on golden hamsters *Mesocricetus aureus* have shown that inability of circadian clocks to entrain has deleterious fitness effects (Hurd and Ralph 1998), and competitive ability in different strains of the cyanobacterium *Synechococcus* has been shown to be a function of how closely the clock period of the strain matched with the period of the imposed light:dark cycle (Ouyang et al. 1998; but see also Woelfle et al. 2004). Latitudinal clinal variation for period and phase of eclosion rhythm in *Drosophila littoralis* and *D. pesudoobscura* (Lankinen 1986, 1993), and for allele frequencies at the period (per) locus in *D. melanogaster* (Costa et al. 1992; Sawyer et al. 1997), has also been

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interpreted as evidence for adaptive significance of circadian clocks (but see also Sharma and Joshi 2002). It has also been suggested that functioning biological clocks may be beneficial to organisms both in terms of maintaining temporal metabolic order internally (Sheeba et al. 1999a), and in phasing one's activities to various ecological factors (Kureck 1979; Daan and Tinbergen 1980; De Coursey et al. 1997; De Coursey and Krulas 1998). Recently, attempts have also been made to model the very early evolution of circadian systems (Roenneberg and Merrow 2002).

In evolutionary biology, on the other hand, the study of life-history evolution has advanced considerably in the last few decades (reviewed by Prasad and Joshi 2003), but the possible relationship between biological clocks and life-history related traits has been addressed in only a few studies (Kyriakou et al. 1990; Miyatake 1996, 1997, 2002; Shimizu et al. 1997; Oklejewicz 2001). Yet, there is good reason to suspect the involvement of circadian clocks in the life-history of organisms. Life-histories are all about the timing of key events in life that have a bearing on the realization of Darwinian fitness through reproduction (Stearns 1992), and biological clocks of circadian periodicity are well known to be mechanisms for keeping time in practically all living organisms (Zordan et al. 2000). Moreover, genetic or environmental manipulations of circadian clock parameters have also been shown to affect many behavioural and physiological traits, including many life-history related traits (Pittendrigh 1993; Sharma 2003b). In *D. melanogaster*, for example, light:dark regime affects traits such as pre-adult development time (Sheeba et al. 1999b), pupation height (Markow 1981; Paranjpe et al. 2004), lifetime fecundity (Sheeba et al. 2001), and longevity (Pittendrigh and Minis 1972; Allemand et al. 1973; Klarsfeld and Rouyer 1998; Sheeba et al. 2000). The evolution of circadian organization has recently been reviewed (Sharma and Joshi 2002; Sharma 2003a) and, therefore, in this article I will restrict myself to reviewing the small number of studies that point toward a role for circadian clocks in determining traits important to life-history evolution, and try to make a case for the importance of incorporating a chronobiological perspective in studies of life-histories and how they evolve. I will discuss mostly studies done on fruitflies of the genus *Drosophila*, because this is a system particularly well studied in the context of life-history evolution (Prasad and Joshi 2003), and is also an increasingly important model system for clock studies on the interface of chronobiology and evolution (Sharma and Joshi 2002; Sharma 2003a,b).

Drosophila life cycle

Drosophila are holometabolous, going through distinct egg, larval and pupal stages before becoming adults. In the most commonly studied species,

D. melanogaster, a well fed uncrowded laboratory population can go from egg to egg in about 10 days at 25°C. Eggs typically hatch in 18-24 hours, and individuals then pass through three larval instars, with the first two instars about 24 hours each in duration whereas the third instar lasts about 48 hours (Bakker 1959). The larval stage is important to the life-history because the size of the adults - which affects female fecundity - is largely fixed by the size at which third instar larvae undergo pupariation.

Early in the third instar, the larvae attain a critical developmental stage marked by a small ecdysone pulse, and a commitment to metamorphosis is made at this point (Berreur et al. 1979). The attainment of this critical developmental stage of 'no return' appears to be correlated with the attainment of a certain critical size/weight which is about half of the final size of a well fed larva prior to pupariation in *D. melanogaster* (Bakker 1959; Robertson 1963). In *D. melanogaster*, it is difficult to alter the duration from the attainment of the critical size/weight till pupariation by changing the nutritional environment, whereas the time taken from hatching to attainment of the critical size/weight is markedly sensitive to nutritional levels and can be lengthened greatly by feeding larvae a sub-optimal diet (Bakker 1959; Robertson 1963). Late in the third instar, a large ecdysone pulse sets the stage for pupariation, which occurs about 5 h after the pulse; another ecdysone pulse about 10 hours after pupariation finally sets into motion a cascade of events leading to pupa formation (White et al. 1997, 1999), and the pupal duration is about 4 days. The duration of the larval stage does not differ between the sexes, but pupal duration in males is about 6 h more than females (Bakker and Nelissen 1963; Nunney 1983), and it is speculated that the longer male pupal duration is due to some aspects of sperm maturation (Nunney 1996).

The division of the larval stage into pre- and post-critical size phases has important implications for the relationship between larval development time and adult size at eclosion, both of which are important life-history characters (Bakker 1959; Robertson 1963; de Moed et al. 1999). It is useful to consider the larval stage as consisting of two distinct processes occurring over time: growth (increase in biomass) and development (a complex series of steps involving hormone mediated changes in gene expression patterns leading to the differentiation of cell types). If growth and developmental rates are at least partly under independent genetic control, then a whole variety of correlated responses to selection for body size or development time would be possible. For example, body size could, in principle, be altered either by changing critical size, thereby leading to a change in development time, or by altering the growth rate in the post critical size period which would not alter the development time (Robertson 1963). Whether critical size or growth rate is affected more by selection on development time or body size has been shown to depend partly on the nutritional environment (Robertson 1963).

After eclosion, adults in lab populations may live 35–40 days, and lifespan trades off with fecundity (Chippindale et al. 1993; Partridge and Barton 1993; but see also Rose and Bradley 1998). Males and females can begin mating 8–10 hours after eclosion, although mean time to first mating is usually between 12–20 hours post eclosion. Females typically start laying eggs within 1–2 days after eclosion, although peak fecundity is not attained till a couple of days after eclosion and mating, and then tends to slowly drop off with age after a plateau in egg laying level that can last for 2–20 days (Novoseltsev et al. 2002).

Many aspects of the life cycle in *Drosophila* are known to be either under circadian control, or affected by the circadian clock. In lepidopterans, the timing of the pre-pupariation hormonal pulse is determined by the clearing of juvenile hormone from the hemolymph, and further subjected to circadian gating, yielding a circadian rhythm in pupariation (Davidowitz et al. 2003), and pupariation does appear to be rhythmic in at least some *Drosophila* species (Bakker and Nelissen 1963; Pittendrigh and Skopik 1970). Eclosion of adults from the pupa in *D. melanogaster* is under control of a circadian clock (Pittendrigh and Skopik 1970; Qiu and Hardin 1996), and in most wild type strains peak eclosion occurs shortly after the dark to light transition. The developmental state of a pupa is assessed once a day through some unknown circadian clock controlled/mediated mechanism, and individuals that have attained a certain developmental state by then will eclose during the next available circadian gate (Qiu and Hardin 1996). In wild type flies, under an LD 12:12 h cycle, the circadian gate is several hours long, starting 1-2 h before the dark to light transition, and the check on developmental status takes place 10h prior to the gate's opening (Qiu and Hardin 1996). Substantial additive genetic variation exists for phasing of the eclosion peak in lab populations of *D. melanogaster*, which readily respond to selection on phase angle difference (V. K. Sharma *pers.* comm.). In addition to eclosion, circadian rhythms are also seen for mating (Sakai and Ishida 2001; Tauber et al. 2003), vitellogenesis (Allemand 1976), and oviposition (McCabe and Birley 1998; Sheeba et al. 2001) in *D. melanogaster*, with the typical pattern of phasing being peak mating activity around the dark to light transition and a subsidiary peak around the light to dark transition (Partridge et al. 1987c; Sakai and Ishida 2001), and peak oviposition shortly after the light to dark transition (Allemand 1976; Sheeba et al. 2001). Indeed, given that over 100 genes involved in a variety of functions including detoxification, olfaction, signaling, conveying nutritional information, cuticle formation and immunity, are now known to be transcribed in a circadian manner under the control of the clock (clk) locus (McDonald and Rosbash 2001), it will not be surprising to find more and more traits relevant to the life-history that are affected to some degree by the circadian clock.

Light:dark regime and Drosophila life-history traits

Traits like pre-adult development time (Sheeba et al. 1999b), lifetime fecundity (Sheeba et al. 2000), adult lifespan (Pittendrigh and Minis 1972; Klarsfeld and Rouyer 1998; Sheeba et al. 2000) and, possibly, larval growth rate (Sheeba 2002) in *D. melanogaster* have been observed to be affected by the imposed light:dark regime. Development time in constant light (LL) is shorter than in a 12 hour light:12 hour dark cycle (LD 12:12), although flies in both light regimes eclose at the same sex-specific dry weight (Sheeba 2002). Pre-adult development time is also affected by light:dark cycles of periodicity other than 24 hours, with development time in LD 10:10 being shorter, and in LD 14:14 being longer, respectively, than in LD 12:12 (Paranjpe et al. 2005). Pre-adult survivorship, however, does not appear to be affected by light regime (Sheeba et al. 1999b; Paranjpe et al. 2005). Fecundity in the first few days of life, and in mid-life (20–30 days post eclosion) is also higher in flies kept in LL rather than LD 12:12, and this appears to be part of the reason for lifespan in LL being shorter than that in LD 12:12 (Sheeba et al. 2000; Sheeba 2002). It is also possible that part of the reason for reduced lifespan in LL is light regime induced arrhythmicity, as virgin females also show reduced lifespan in LL compared to LD 12:12 (Sheeba et al. 2000). Pupation height - the height above the medium at which larvae pupate - is also affected by light regime, with pupation height being the greatest in constant darkness (DD) and LD 10:10, intermediate in LL, and lowest in LD 12:12 and LD 14:14 (Paranjpe et al. 2004), suggesting that the higher pupation height observed in DD compared to LL is not merely due to inhibition of larval wandering by light, as we earlier hypothesized (Markow 1981), but has some involvement of the circadian clock. Given that different light:dark regimes are known to affect circadian clock parameters (Pittendrigh 1993), and that we also have some understanding of the molecular underpinnings of the circadian clock, especially in *D. melanogaster* (Sharma 2003b), there is clearly a need now for studies aimed at understanding the possible role of circadian clocks in determining different life-history related traits.

Circadian clocks and Drosophila life-history traits

Pre-adult development time is the most obvious life-history trait in *D. melanogaster* that might be strongly affected by the circadian clock, because eclosion is subject to circadian gating; development time is also the main life-history related trait that has been studied in some detail in this context. Moreover, development time is among the major life-history traits whose evolution has been extensively studied in laboratory populations of *Drosophila melanogaster,* particularly with regard to how development time trades off with other life-history traits (Zwaan et al. 1995; Nunney 1996; Chippindale

et al. 1997; Prasad et al. 2000, 2001). There are two lines of evidence supporting the notion that the circadian clock, or at least the eclosion rhythm, may be involved in determining development time in insects. A study of per^S and per^L mutants of *D. melanogaster,* that have free-running periods of eclosion rhythm that are, respectively, shorter and longer than that of the wild-type flies, revealed a positive correlation between free-running period and pre-adult development time under both dim LL and DD (Kyriakou et al. 1990). However, the development time differences between the three lines also persisted in bright LL, a regime in which eclosion is arrhythmic (Konopka 1972), suggesting that the effects of *per* alleles on development time might be through a pleiotropic effect not mediated via the eclosion rhythm per se (Paranjpe et al. 2005).

In a set of studies on the melon fly *Bactrocera cucurbitae*, Miyatake and colleagues showed that development time was positively correlated with the phase of mating rhythm (longer development line flies mated later in the night), and the free-running period of the locomotor activity rhythm, across populations selected for shorter or longer development time (Miyatake 1996, 1997; Shimizu et al. 1997). However, selection was successful only for longer development time, and only the longer development lines diverged in correlated circadian rhythm traits from the ancestral population. There were also large differences in mean phenotype between the two replicate longer development lines (Miyatake 1997). Population sizes were also quite small ($N = 100$): all these facts together make it difficult to rule out inbreeding/genetic drift and selection for generally bad genotypes (as a consequence of selecting for longer development i.e. for lowered fitness) as an alternative explanation for their results. In a more recent study, lines of *B. cucurbitae* selected for reproduction at early or late ages were seen to diverge in phase of mating rhythm and in period of locomotor activity rhythm, with flies from the early reproducing lines mating earlier in the day and showing a shorter period of locomotor activity rhythm than flies from the late reproducing lines (Miyatake 2002). The interpretation of these results, too, is not unequivocal because the early reproducing lines also evolved shorter development time, possibly as a response to inadvertent selection for rapid development through a benefit to be gained from a slightly longer maturation time between eclosion and the age of early reproduction (Miyatake 2002). Interestingly, in a very different model system, Syrian hamster (*Mesocricetus auratus*) mutants at the *tau* locus that shortens the period of the locomotor activity rhythm, have been found to differ in metabolic rate, growth rate and lifespan from wild type individuals (Oklejewicz 2001).

In general, we can make two hypotheses about the role of circadian clocks in determining development time in *D. melanogaster*, based on whether subjective time (biological clock time) or objective time (external time) governs

the developmental process. One hypothesis is that developmental processes scale to internal or biological clock time. If so, the development time for a population should be a multiple of the period of the biological clock, plus some additional time determined by the phasing of the eclosion gate. In the second hypothesis, the developmental processes are assumed to be determined by real time (external time based on the earth's rotation). If so, the development time for a population should be fixed in hours, plus some additional time determined by the phasing of the eclosion gate. Another way of looking at these hypotheses is that in the first, it is the biological clock that times eclosion, whereas in the second the biological clock merely determines the time of day that peak eclosion occurs. These two hypotheses represent the extremes of a spectrum of possible clock control of development time in insects.

We have recently tested these hypotheses by studying development time of *D. melanogaster* populations under different light:dark regimes (Paranjpe et al. 2005). The period of the eclosion rhythm can be altered by changing the period of the imposed LD cycle, and the eclosion rhythm in *D. melanogaster* populations in our laboratory entrains to imposed light:dark regimes of LD 10:10, LD 12:12 and LD 14:14 (Paranjpe et al. 2003). We measured pre-adult development time in four populations of flies under LL and DD (in both of which the free-running period of the clock is expressed, although free-running periods in LL and DD are different), and LD 10:10, LD 12:12, and LD 14:14. Entrainment implies that in the three LD regimes the period of the biological clock is 20, 24 and 28h, respectively. From the data on eclosion rhythm period, and phase of eclosion in these five regimes, expected development times can be derived under both the hypotheses outlined above, and compared to observed data. The observed development times in the five light:dark regimes were not consistent with predictions under either of the two hypotheses, indicating that although the eclosion rhythm, if not clock, does play a role in determining development time beyond its role in timing the eclosion gate to a specific part of the day, the relationship between clock period and development time is also not as simple as the latter being a multiple of the former. This view is supported by the observation that circadian clocks are expected to show fairly strong temperature compensation (Pittendrigh 1960), whereas life-stage duration is markedly affected by temperature in ectotherms, including *Drosophila* (David et al. 1983). Indeed, even the clock control of the eclosion rhythm may be more complex than previously thought: it has recently been shown that a peripheral clock in the prothoracic gland is required, in addition to the main clock in the lateral neurons, for the proper expression of the eclosion rhythm in *D. melanogaster* (Myers et al. 2003).

The importance of a chronobiological perspective in life-history evolution

"waqt se din aur raat, waqt se kal aur aaj
waqt ki har shai ghulaam, waqt ka har shai pe raaj"

"Time determines now and then, and the cycling day and night
On every aspect of existence, Time does exercise its might"

(Sahir Ludhianvi, urdu poet: translation by the author)

Time, most directly in the form of a 24 hour periodicity due to the earth's rotation, is an extremely important aspect of the environment for any living organism, and circadian clocks are a fundamental adaptation to life on a rotating planet (Sharma 2003a,b). The impact of circadian organisation on traits important to the life-history is, therefore, likely to be both pervasive and important. However, as summarised in this article, not too much is as yet known about this important area in our understanding of organismal biology. We have seen that there is at least strongly suggestive evidence for circadian clocks mediating, if not controlling, key life-history traits, as well as the possibility that some evolutionary responses of life-history traits to selection may be partly mediated through changes in clock characteristics. There is also preliminary evidence for laboratory evolution of circadian organisation in response to maintenance in different light:dark regimes, although it is not as yet clear what the light regime-specific life-history correlates of these changes in circadian rhythm parameters are (Sheeba 2002). Overall, although the evidence is as yet patchy, it does seem that circadian organization plays a role in determining the timing of life-history events, albeit perhaps a subtle one. I believe this is likely to prove an important area for future studies of the physiological and ontogenetic shaping of traits underlying adaptive evolutionary changes in Drosophila life-history.

Another aspect of how a chronobiological perspective is likely to be helpful in studies of life-history evolution pertains to the design of experiments. Laboratory studies with *D. melanogaster* have been variously conducted under LL, LD 12:12, LD 16:8, and sometimes even under fluctuating LD regimes wherein the timing of lights on and off is completely arbitrary, and based on when people enter or leave the laboratory. Often, the light:dark regime used in *Drosophila* life-history evolution studies is not even mentioned in published papers. As we have seen, light regime affects most life-history traits in *D. melanogaster*, including pre-adult development time, fecundity and lifespan. There may well be other ways in which light regime interacts with selection in mediating correlated responses of which we are as yet unaware, and this is an area that deserves close attention when designing experiments. It is worth

noting in this context that many of the inconsistencies between laboratories in correlated responses to selection on age-specific fecundity and lifespan (Ackermann et al. 2001) are between laboratories using LL and LD 12:12, respectively, as the rearing light regime. In conclusion, I would like to stress that I believe it is time for life-history evolution studies to take greater cognizance of the ubiquity of circadian phenomena in living systems, and their sensitivity to light:dark regimes, in the context of both the design and the interpretation of experiments.

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