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Exceptionally short-period circadian clock in *Cyclosa turbinata*: regulation of locomotor and web-building behavior in an orb-weaving spider

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Abstract. A major advantage of having behavior controlled by a circadian clock is that the organism may be able to anticipate, rather than respond to, important daily events in its environment. Here, we describe the behavioral rhythms of locomotor activity and web building in the orb-weaving spider *Cyclosa turbinata* (Walckenaer, 1841). Web building occurs late in the scotophase, in absolute darkness, and is initiated and completed before lights-on under light:dark cycles in the laboratory. This scheduling presumably enables web-building to occur under the cover of darkness, thereby avoiding visual predators. Locomotor activity occurs predominantly in the dark with a sharp peak within one hour after lights-off and a broader peak occurring before lights-on. The locomotor activity rhythm free runs under constant dark and constant temperature conditions, thus indicating endogenous circadian control. Evidence from the free running rhythm suggests that the first peak under light:dark cycles is a result of masking but that the second peak is attributable to the endogenous circadian oscillator. The period of the free run is exceptionally short, about 19 hours. In comparison with locomotor activity, web building is quite sporadic under constant dark conditions, making detection of periodicities difficult and, therefore, whether web-building is under endogenous circadian control or is driven by exogenous factors remains unresolved.

Keywords: Behavioral rhythm, chronoecology, locomotor activity, adaptation

It is commonly assumed that circadian rhythms are adaptive, enabling the organism to perform each of its various functions, including behavior, at the most appropriate time of day with respect to its environment. In many cases, the possession of a circadian clock empowers the organism with the ability to anticipate important daily events in its ecosystem, such as the occurrences of food, potential mates, and predators. In the flying squirrel, for example, the circadian clock controls the emergence of this nocturnal animal from its den shortly before dark, allowing the squirrel to begin its activity in accord with a particular light intensity after sunset (DeCoursey 1989). Forager honey bees rely on a continuously-consulted circadian clock that allows them to remember the time of day of previously productive floral food sources (Beling 1929; Wahl 1932), thereby providing a mechanism to match foraging behavior with nectar secretion rhythms. A prominent feature of this honey bee 'time memory' is that foragers typically anticipate the previously rewarded time of day by making reconnaissance flights to the food source (Moore & Doherty 2009). Because the initial encounter of a forager with a new nectar source may not occur at the onset of a particular flower species' nectar availability, early investigative flights on subsequent days may be adaptive by scheduling foragers to arrive earlier within the window of opportunity (Van Nest & Moore 2012). More direct support for the adaptive significance of circadian rhythms is provided by Spoelstra et al. (2016) who showed that mice with short period circadian rhythms (via the *tau* mutation) released into outdoor enclosures showed reduced survivorship and reproduction relative to wild-type animals and Woelfle et al. (2004) who demonstrated that cyanobacterial strains with functioning circadian clocks outcompeted clock-disrupted strains in rhythmic environments.

For any spider, there is a precarious balancing act between the need to be aggressive enough to capture prey, yet wary enough to avoid predation. The relative expression of these two opposing behavioral states is expected to vary in accordance with daily changes in environmental conditions. Orb-weaving spiders are well-established models for ecological studies including consequences of habitat choice (reviewed by Riechert & Gillespie 1986), prey capture efficiency (reviewed by Eberhard 1986), and group foraging (reviewed by Uetz & Hieber 1997). Orb-weaving spiders have different temporal foraging patterns across species. Most are nocturnal, building their webs in the evening and foraging through the night, then leaving the web for a hideaway during the day. In *Larinioides cornutus* (Clerck, 1757), a night-foraging orb-weaver, there is a circadian clock-controlled partitioning of the antipredator 'huddle response' such that spiders are significantly more defensive during the day than during the night (Jones et al. 2011). Some species build webs and forage diurnally, while other species forage continuously around-the-clock. However, it is widely believed that the reason most orb-weaving species are nocturnal is to avoid diurnal predators such as birds and wasps (Cloudsley-Thompson 1958). In fact, many of the relatively few diurnal species have adaptations to avoid bird predation, such as abdominal spines or foul-tasting guanine deposits (Cloudsley-Thompson 1995). While reduced exposure to predators may be an advantage to the nocturnal behavioral phenotype, there may be associated costs including reduced prey density at night, shortened foraging periods in summer months, and increased competition with other spiders (Wise 1993). Continuous foragers perhaps may be so prey-limited that they are forced to forage around-the-clock.

Cyclosa turbinata (Walckenaer, 1841) is a small (4–7 mm) orb-weaving spider which forages in its web both day and night. Prey carcasses are incorporated into the web, forming a "trashline" which functions as daytime camouflage to avoid visual predators such as birds and wasps (Tseng & Tso 2009; Gan et al. 2010). While the trashline attracts attacking wasps, they are usually unable to locate the spider resting upon it (Chou et al. 2005). For orb-weaving spiders, the timing of daily web-replacement is particularly important because the web's prey capture effectiveness declines with the passage of time after construction (Foelix 1996) and movements associated with web replacement may attract visual predators (Rypstra 1982). Although prey may land on the web at all times of day, data from lakeside habitats suggest that prey abundance increases over the course of the afternoon and peaks early in the night (Watts et al. 2015). In webs constructed under laboratory conditions, *C. turbinata* individuals exhibit the highest levels of foraging aggression during the night and early morning. Surprisingly, this behavioral state coincides with the time of day in which the spider has the highest probability of retreating from simulated predator attacks (Watts et al. 2014). The lowered probability of retreating during most of the daylight hours suggests a greater reliance on a passive antipredator strategy (remaining immobile, using the trashline as camouflage) compared to the more active strategy of fleeing from the web hub or dropping from the web. Taking into account all of these behavioral tendencies, we hypothesize that *C. turbinata* schedules its daily routine so as to replace its web under the cover of darkness, allowing enough time for completion of the building process before dawn, yet close enough to dawn so as to maintain the web's capture effectiveness as prey density increases over the course of the day.

The primary objective of the present study is to describe the exact timing of web replacement behavior in *C. turbinata* with respect to light:dark cycles and then to determine the mechanism by which this temporal phasing is controlled. Spiders have been shown to rely on circadian clocks to regulate a variety of behaviors and physiological processes (Seyfarth 1980; Schmitt et al. 1990; Suter 1993; Yamashita & Nakamura 1999; Ortega-Escobar 2002; Jones et al. 2011). However, current evidence from nocturnal orb-weavers under laboratory (Ramousse & Davis 1976) and natural (Ceballos et al. 2005) conditions as well as from a diurnal orb-weaver during a total solar eclipse (Uetz et al. 1994) suggests that web-building may respond directly to light conditions, rather than be controlled by an endogenous, light-entrainable circadian rhythm. If the phasing of web replacement behavior is under circadian clock control, then the diel behavioral rhythm should continue (free run) with the period of the underlying clock under constant conditions. Alternatively, if web replacement behavior is controlled exogenously *(*i.e., it responds directly to changes in light levels), then web-building behavior should occur immediately (or shortly) after dusk or dawn, and the diel rhythm, absent the need for circadian control, should not persist under constant dark conditions. In order to interpret these patterns in an ecological context, we also quantify diel patterns of prey and threat abundance in typical *Cyclosa* habitat.

METHODS

Study species.—Adult female *Cyclosa turbinata* (Araneae: Araneidae), commonly found along the edges of forested areas in the southeastern United States, were collected in Washington County, Tennessee in 2012, 2013, and 2015. Spiders were collected with their webs and trashlines and maintained in plastic deli containers. Care of the animals followed ASAB/ABS guidelines, and the animals were released following experiments.

Locomotor activity.—Locomotor activity was recorded continuously. Individual spiders were placed in 25 mm diameter X 100 mm length, clear plastic tubes and inserted into a locomotor activity monitor (model LAM) from Trikinetics, Inc. (Waltham, Massachusetts). Activity within each tube was measured via interruption of three infrared beams transmitting through the center of the tube: each interruption was registered as an event. To minimize possible visual interactions among the spiders placed in close or adjoining positions, the tubes containing the spiders were painted opaque white along their lengths, except for a small, central band allowing for transmission of the infrared light. Screen was fastened to each end of the tube and, on one end, water was available ad libitum via a cotton-plugged, flexible tube (10 mm diameter) projecting through the screen. Events were counted in 6-min bins and analyzed using Clocklab Analysis Software (Actimetrics, Wilmette, IL, U.S.A.). Activity was depicted graphically by double-plotted actograms to facilitate visual recognition of periods. Significant periods were detected using two different periodogram analyses, chi-square and Lomb-Scargle. The chi-square periodogram (Sokolove & Bushell 1978) is broadly applicable for analyzing circadian data. The Lomb-Scargle periodogram, using a form of Fourier spectral analysis, is better suited to analyze records with large or frequent gaps (Van Dongen et al. 1999). The use of these two complementary methods, rather than just one, provides additional support for determinations of period. Because of the sparse nature of activity bouts exhibited by the spiders, especially with respect to web-building behavior, we accepted circadian periodicities only if indicated by both methods.

Two different sets of spiders were tested. The first set was captured in August 2012. These spiders were monitored in a temperature-controlled environmental chamber (25 ± 0.5 °C) under a light-dark cycle containing 12 hours of light and 12 hours of dark (LD 12:12) for five days and then under constant darkness (DD) thereafter. Lights-on occurred at 08:00 h and lights-off at 20:00 h. Light during photophase was provided by four vertically mounted, 32 W fluorescent tubes and the illuminance was approximately 1400–1600 lux at the level of the activity monitor. Spiders from the second set were captured in August 2015, entrained under a ramping light:dark cycle (see below) for two weeks in the laboratory at 25 °C, the last two days of which were in the activity monitor. Recordings started on day 2 of constant dark conditions. Unlike the spiders from the 2012 collection, these spiders were not provisioned individually with water tubes but were housed in a laboratory room under high humidity conditions.

Web-building activity.—To determine the phasing of web-building behavior with respect to a light:dark cycle, individuals were maintained in 30 × 30 x 10 cm wooden frames (Watts et al. 2014) in the laboratory under either a hard (N = 8) or ramping (N = 10) LD 12:12 h cycle. The ramping cycle was employed as a more accurate simulation of natural conditions. For the hard LD 12:12 cycle, lights-on occurred at 07:00 h and lights-off at 19:00 h. For the ramping LD cycle, the dark-to-light transition began at 07:00 h and was completed at 08:00 h; the light-to-dark transition likewise took place over one hour and began at 19:00 h. Therefore, in the hard LD cycle, spiders were exposed to light (at a continuous 1000–1200 lux) for 12 hours. In the ramped LD cycle, spiders were exposed to continuous light (1000–1200 lux) for 11 hours, flanked at the beginning and end with 1-hour periods of gradually increasing or decreasing light levels.

Spiders used in the hard LD 12:12 cycle were collected in August 2012 and maintained individually in 110 ml plastic "deli" containers for 5 days under LD 12:12 conditions. They were then moved to the wooden frames and the frames were wrapped in clear plastic food wrap, enabling web construction to occur overnight. On the day after transfer to the frames, the plastic wrap was removed and each spider was fed two *Drosophila hydei*. Behavior was then video-recorded at 6 frames/s for the next 5 days under the same light:dark cycle at 23 °C using surveillance cameras equipped with infrared night vision (Q-SEE QT9316 and Defender SP-301).

Spiders used in the ramping LD 12:12 cycle were collected in July and August of 2013 and were transferred directly to the wooden frames or kept in deli containers. Spiders not building a web within two days were replaced with spiders from the deli containers. All web-building spiders were acclimated to the ramping LD 12:12 cycle for at least 5 days and then tested in their webs for their foraging and antipredator behavior (Watts et al. 2014) from 11–21 August 2013. On 21 August, the spiders were misted with water and fed two termites. On the following day, they were misted but not fed. Then, on 23 August (day 1 of the experiment), the behavior was video-recorded at 6 frames/s under the ramping LD 12:12 cycle at 23 °C for 5 days and then for 12 days under constant dark conditions. The spiders were misted and fed two termites every three days (thereby preventing entrainment to the feeding event), beginning on day 4. Web-building activity (either present or absent) was binned in 6-min increments and analyzed, as with locomotor activity, with ClockLab Analysis Software.

Diel patterns of prey and threat density.—We used a malaise trap (Bugdorm SLAM Trap- Standard 110x110x110 cm) to sample flying insect densities over the diel cycle. The trap was placed in typical *Cyclosa* habitat along a mowed path between an old field and shrubline, in Washington County, Tennessee, USA. The contents of the trap were emptied every three hours, and trapping continued for three consecutive days in June 2014 during which there was no rain. Between 24-hour cycles, the trap was moved to a new location 25 m along the path. The collected insects were identified to order, and their body lengths were measured to the nearest mm. While there are no data on specific predators of *C. turbinata*, Chou et al. (2005) found that wasps frequently attack other species of *Cyclosa*. Thus, we considered wasps to be potential threats, and non-wasp insects under 15 mm to be potential prey.

RESULTS

Locomotor activity.—Thirteen *C. turbinata,* captured in 2012, performed locomotor activity though all 5 days of the LD 12:12 cycle. We calculated DiNoc ratios as: daytime activity – nighttime activity/ total activity (Suter & Benson 2014). All of the spiders' ratios were below 0 (mean = –0.79, SD = 0.26) indicating predominantly nighttime activity, with two showing exclusively nighttime activity. The entrainment profile (Fig. 1) illustrates this nocturnality plus several additional trends. First, locomotor activity had a prominent peak occurring within one hour after lights-off, followed by a gradual build-up in activity through the scotophase, reaching a second, broader peak at about 05:00. The activity then declined through the remainder of the scotophase, extending into the first 4 h of the photophase, interrupted briefly by a sharp peak occurring immediately after lights-off. There was absolutely no activity during the last 8 hours of the photophase.

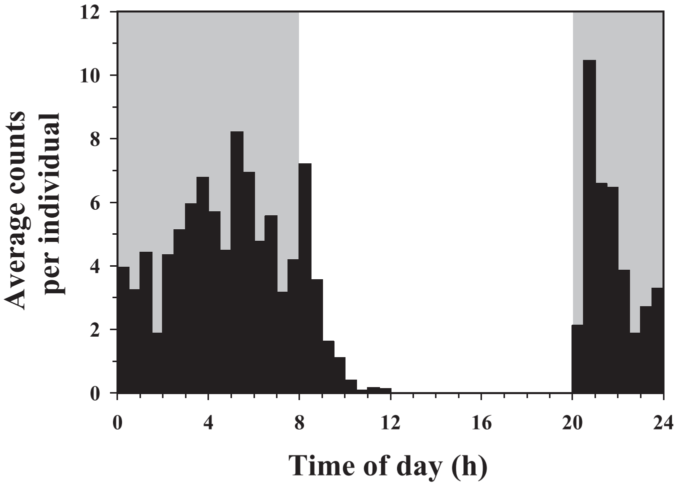

Figure 1.—Entrainment profile for locomotor activity in *C. turbinata*, illustrating average counts (interruptions of the infrared beam) per individual throughout the day, in 30-min intervals, for the last four days of the LD 12:12 h cycle. Dark background indicates dark portion of LD cycle.

Ten spiders continued to show locomotor activity under constant dark conditions for at least 5 days after discontinuation of the LD cycle. Nine of these exhibited a robust, free-running rhythm with a significant and consistent period as revealed by chi-square and Lomb-Scargle periodogram analyses (Table 1). The mean free-running period, based on the chi-square periodograms, was 18.74 ± 0.13 h (SEM) for these spiders. In one case (Ct-10, Table 1), the periodogram analyses yielded significant, but different periods and, therefore, this individual was not used in the determination of mean free-running period. In another individual (Ct-13, Table 1), the chi-square periodogram indicated two significant periods (28.8 and 19 h) and the Lomb-Scargle identified only one (19.1 h). Because it was detected by both analyses, we accepted the 19 h rhythm in our calculation of mean free-running period.

Actograms and accompanying periodograms (Fig. 2) illustrate the nocturnality under the LD 12:12 h cycle and the extremely short endogenous periodicities of locomotor activity expressed by individual spiders under DD conditions. In individual Ct-17 (Fig. 2a), there is a predominance of activity during the scotophase of the LD cycle, especially during the second half of the night. Under DD, this spider exhibited a very short circadian rhythm of about 18.8 h, confirmed by the chi-square periodogram (Fig. 2a). The activity performed during each circadian cycle was restricted

Table 1.—Chi-square and Lomb-Scargle periodogram analyses of locomotor activity rhythms under DD. Indicated are the free-running periods under DD conditions (τ_{DD}), in hours, that were significant at $\alpha = 0.001$ (with two exceptions at $\alpha = 0.05$, indicated in parentheses). The first 10 individuals were captured in 2012; the last 5 in 2015.

	τ_{DD}	
Identity	chi-square	Lomb-Scargle
Ct-03	19	18.75
Ct-06	18.9	19.1
Ct-07	17.8	18.1
Ct-08	19	19.55
Ct-10	31.2	15.7
Ct-13	19, 28.8	19.15
Ct-14	18.4	18.65
Ct-16	18.8	18.75
Ct-17	18.8	18.8
Ct-19	19	18.7
CtF15-M1	19.8, 26.6	27.2 (0.05)
CtF15-M2	17.9	18.4
CtF15-M3	19	—
CtF15-M4	20	—
CtF15-M7	17.5	18.85 (0.05)

to short bouts of activity, ranging from about 1–4 h in duration. Individual Ct-14 exhibited sporadic, predominantly nocturnal activity under the LD 12:12 h cycle and a very short circadian rhythm of about 18.4 h in DD (Fig. 2b). Extrapolation back from the sequential onsets of activity in both actograms indicates that the free-running activity originated in late scotophase, suggesting that the nocturnal activity during this phase is under circadian control.

The free-running locomotor rhythms of an additional five *C. turbinata*, captured in August 2015, were recorded in the activity monitors under constant dark conditions at 25 °C in the laboratory (Table 1). Although all five showed significant periods in the range of 17.5 to 20 h according to chi-square periodograms, only two (CtF15-M3 at 17.9 h and CtF15-M7 at 17.5 h) were confirmed as significant by similar periods (18.4 and 18.85 h, respectively) according to the Lomb-Scargle method (Table 1).

Web-replacement behavior.—The mean onset time for the first component of web-building behavior, radial thread maintenance, occurred at 03:58 h ± 8 min (SEM) under the hard LD 12:12 cycle and 04:41 h ± 16 min (SEM) under the ramping LD 12:12 cycle (Fig. 3). These onset times were significantly different (T-test, $t = -2.31$, $df = 28$, $P = 0.029$). For the second component of web building, sticky spiral replacement, the mean start times under the hard (04:55 h ± 7 min) and ramping (04:59 h ± 11 min) LD cycles were not significantly different (T-test, $t = -0.32$, $df = 31$, $P = 0.751$).

The length of time required to complete both components of web building, for individuals that completed both components, was 118.9 ± 9.5 min (SEM) and 84.5 ± 7.9 min (SEM) under the hard and ramping LD cycles, respectively. Overall, the mean duration of web-building behavior was 103.6 ± 6.9 min (SEM).

Analyses of web-building behavior at the level of the individual spider were accomplished by the use of actograms depicting the occurrence or absence of web-building behavior (both radial thread maintenance and sticky spiral replacement) compiled in 6-min bins throughout the day for the duration of the experiment. Of the eight spiders exhibiting web-building behavior during DD, five performed this behavior only twice during the 12 days of DD and, therefore, were not used for determinations of circadian period. The remaining three individuals provided temporally sparse records under both LD and DD, illustrated by the actograms. For example, spider Ct-web 5 rebuilt its web (in late scotophase) only on days 2 and 3 during the five days of LD entrainment but reconstructed its web on all but one day under DD (Fig. 4a). This activity showed three significant periods (19.6, 26.1, and 29.2 h) according to the chi-square periodogram during the 12 days of DD but different periods were indicated by the Lomb-Scargle method (Table 2). Similarly, spider Ct-web 8 rebuilt its web only once during LD entrainment (again, during late photophase) but skipped only two days during its 12-day tenure under DD (Fig. 4b). Periodogram analyses (Table 2) confirmed three significant periods (18.5, 23.8, and 27.8 h). A third individual, Ct-web 7 (not shown), in accord with the previous two examples, showed web-building behavior on just one day under LD but on 10 days under DD. Periodogram analyses indicated two (chi-square) or three (Lomb-Scargle) significant periods for this activity under DD (Table 2).

Diel patterns of prey and threat density.—In the pooled three days of malaise trap sampling of potential prey and threats (Fig. 5), the distributions of trapped insects were clearly not random and were concentrated in the photophase (prey Chi-square = 131.2, d.f. = 7, $P < 0.001$; wasp Chi-square = 20.5, d.f. = 7, $P = 0.005$). In general, there were more potential prey trapped than potential threats. Also, the diel pattern of prey trapped showed a smooth oscillation over the diel cycle being lowest in the 3 hrs before and after dawn and peaking in late afternoon. There were no potential threats trapped between midnight and dawn and the majority were trapped between 0900 and dusk.

DISCUSSION

Observations under both hard and ramped LD cycles confirm that web-building behavior (consisting typically of both radial thread maintenance and sticky spiral replacement) is accomplished in complete darkness. Initiation of the behavior occurs approximately three hours before lights-on in the hard LD cycle and about two hours, 20 min before the beginning of the upward ramp in the ramped LD cycle (Fig. 4). The significantly later onset of activity in the ramped compared to the hard LD cycle suggests that entrainment of the daily rhythm of web replacement may be accomplished by exposure to a relatively high light level associated with 'dawn'. This exposure presumably would occur toward the end of the upward ramp (near 08:00 h) in the ramped LD cycle and at the lights-on transition (07:00 h) in the hard LD cycle. Because the behavior lasted, on average, about 119 min in the hard LD cycle and 85 min in the ramped LD cycle, the spider's web-building activity is completed before dawn. This scheduling in anticipation of sunrise ensures that movements associated with web-replacement are completed before visual predators are able to detect them (Fig. 5b). During the day, it remains motionless on its web, camouflaged by its trashline. This has

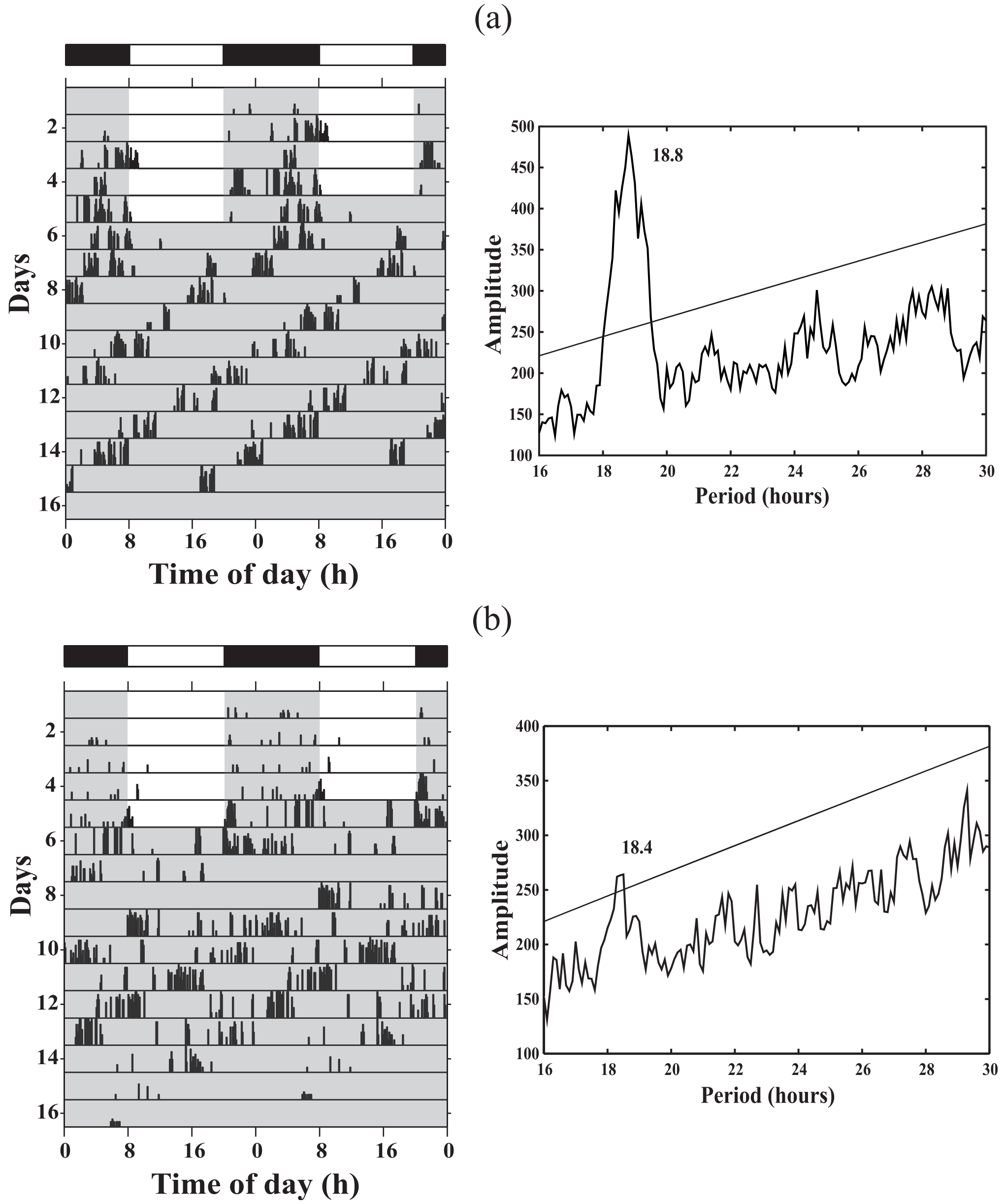

Figure 2.—Double-plotted actograms (left panels) depicting timing of locomotor activity for two individuals (a & b) under hard LD 12:12 h cycle for five days, followed by constant darkness. Dark periods are indicated by gray background. Chi-square periodograms (right panels) indicate significant ($P < 0.001$) periodicities in locomotor activity in constant darkness.

been demonstrated to be an effective antipredator strategy in other *Cyclosa* species (Chou et al. 2005; Tseng & Tso 2009). Its total behavioral commitment to visual crypsis is further supported by experiments showing that its lowest probability of retreating from a predator, as well as its lowest levels of foraging aggression, occur during the daylight hours (Watts et al. 2014). In contrast, another nocturnal orb-weaving spider, *Eriophora edax* (Blackwall, 1863), spins its web immediately after sunset, captures and feeds on its prey exclusively during the night, and dismantles its web just before dawn (Ceballos et al. 2005). Furthermore, because sticky spiral replacement in *C. turbinata* occurs near the very end of the night before sunrise, the web is able to retain its effectiveness (Foelix 1996) through the course of the daylight hours and, therefore, the spiders take advantage of increasing prey density over the course of the day, as found in this study (Fig. 5a) and a previous study (Watts et al. 2015). An additional potential advantage of scheduling web replacement before dawn is that this allows *C. turbinata* to forage in the hours immediately after dusk when prey are still relatively abundant (an advantage not available to spiders which use dusk as a cue to replace the web).

Although web-building behavior under LD conditions occurs during the late scotophase, in complete darkness and in anticipation of dawn (Fig. 3), the sparse actogram records during DD (Fig. 4) provide limited evidence that the behavior is under circadian control. Only three of eight spiders subjected to 12 days of DD performed web-building behavior more than twice. All three of these individuals built webs on at

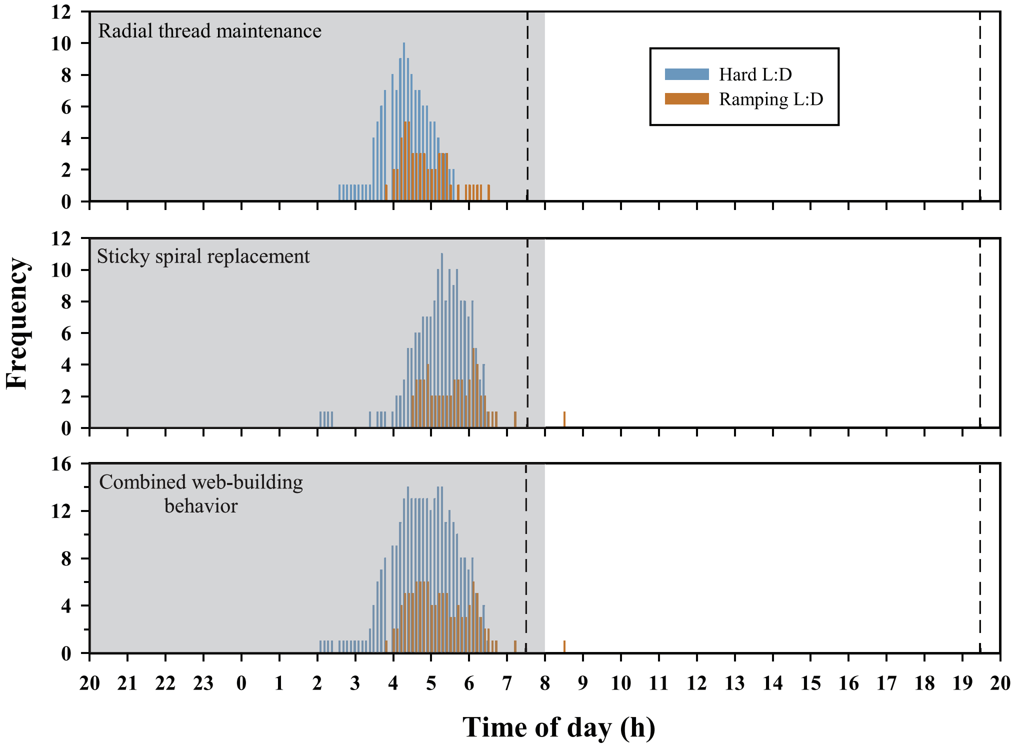

Figure 3.—Histograms depicting pooled frequencies of web-replacement behaviors across times of day for spiders observed under hard and ramping LD 12:12 h cycles. Top panel: radial thread maintenance; middle panel: sticky spiral replacement; bottom panel: combined web-building behavior. Vertical dashed lines indicate the beginning of the light ramp under ramped LD conditions.

least 10 days during DD. For each of these individuals, the chi-square and Lomb-Scargle periodogram analyses revealed at least two different significant periods for the behavior. Agreement between the two periodogram methods was achieved for only two individuals, Ct-web 7 and Ct-web 8 (Table 2). However, one of the significant periods indicated by the periodogram analyses for each of these two spiders (18.1 and 18.5 h, respectively) was in close agreement with the mean period of about 18.7 h exhibited by the locomotor activity rhythms. The absence of a consistent web-building rhythm under DD in *C. turbinata* is not understood. Although the results from two individuals suggest control by a circadian oscillator with a period similar, if not equal, to that controlling locomotor activity, there are other possibilities. For example, coupling of the behavior to the circadian oscillator may exist but be relatively weak. Perhaps many individuals are not inclined to perform web-building activities unless there is some damage to the web. These two possibilities are not necessarily mutually exclusive. Long-term video recordings over a span of several weeks, using a large number of spiders, may be necessary to confirm (or reject) the existence of circadian control of web-building behavior.

The pattern of locomotor activity elicited by *C. turbinata* in response to LD 12:12 conditions (Fig. 1) appears roughly bimodal with a sharp peak occurring within about 1 h after the lights-off transition followed by a more gradual build-up in activity that peaks about 3 h before the lights-on transition. Activity gradually declines from this second peak, except for a sharp increase in activity immediately after the lights-on transition, until terminating completely within the first 4 h of the photophase. Actograms depicting locomotor activity under LD 12:12 followed by DD show a free-running rhythm for which the activity onsets extrapolate back to the end of the scotophase (Fig. 2). These results suggest that the activity at the end of the scotophase may be under circadian control but the activity at the beginning of the scotophase may be the result of extensive masking in direct response to the LD cycle rather than entrainment of the endogenous oscillator. This phenomenon is reminiscent of the locomotor activity rhythm in *Drosophila pseudoobscura* in which there is both a dawn and a dusk peak of activity under LD cycles (Engelmann & Mack 1978). However, under DD, only the dusk peak persists and the resulting free-running rhythm extrapolates back to the phase of the dusk activity peak under LD. The dawn peak presumably is an exogenous response to lights-on. Similarly, the early scotophase peak of locomotor activity in *C. turbinata* may be an exogenous response to the lights-off transition. Further experiments with *C. turbinata* are planned to better characterize the mechanism of entrainment in this nocturnal spider, including determinations of the relative influences of the dawn and dusk transitions, the limits of entrainment, and the relationship between the free running period and phase angle.

The exceptionally short period (about 19 h) of the free-running rhythm of locomotor activity in *C. turbinata* is remarkable. The 19-h period is comparable to the *tau* mutant in hamsters, with a period of about 20 h (Ralph & Menaker 1988), the *per*^{S} mutant in *Drosophila*, with a period of about 19 h (Konopka & Benzer 1971), and the "super duper" mutant in hamsters, showing a period of about 18 h (Monecke et al.

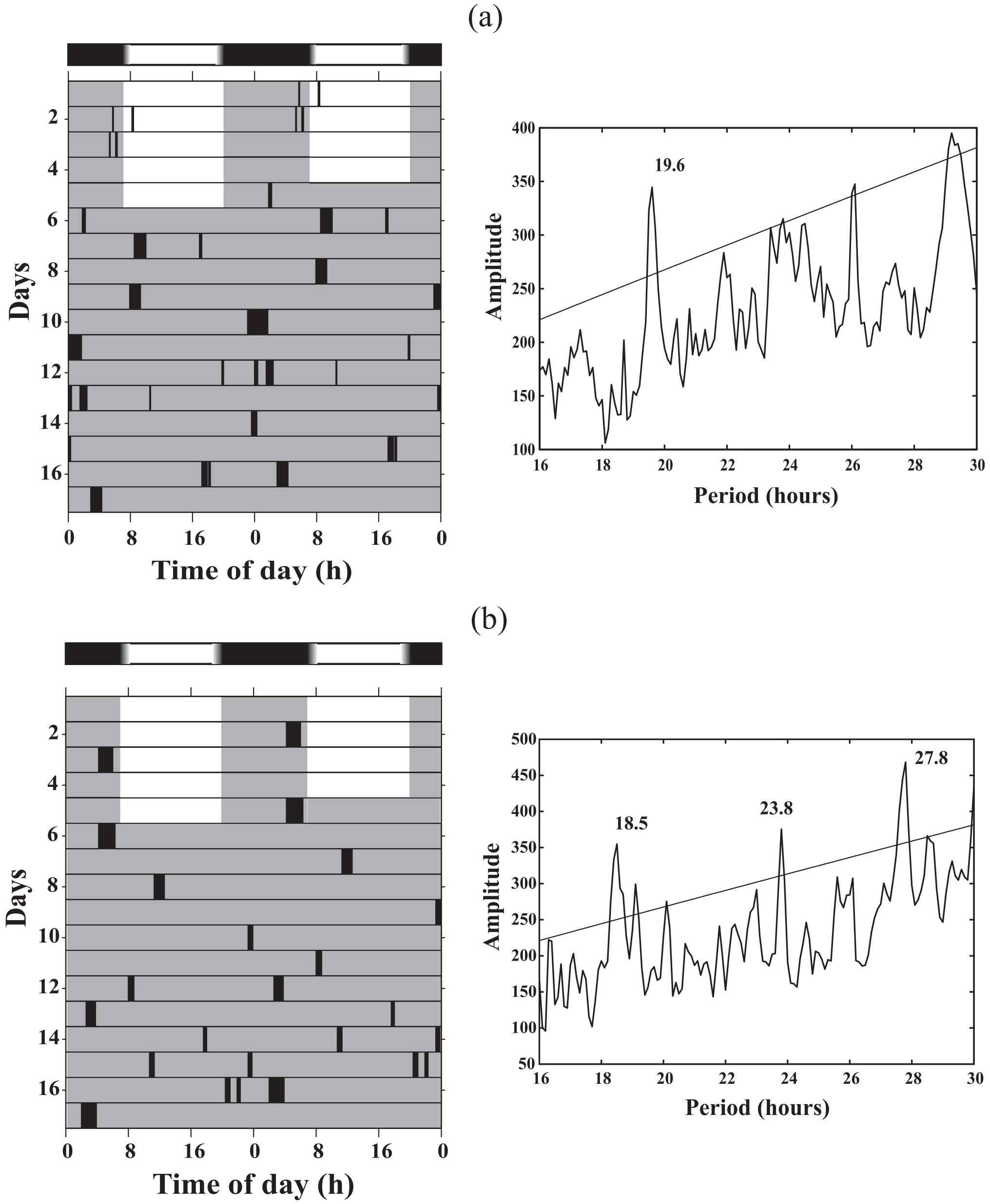

Figure 4.—Double-plotted actograms (left panels) depict timing of web-replacement behavior for two individuals (a & b) under ramping LD 12:12 h cycles for five days, followed by constant darkness. Dark periods are indicated by gray background. Chi-square periodograms (right panels) indicate significant ($P < 0.001$) periodicities in web-building activity in constant darkness.

Table 2.—Chi-square and Lomb-Scargle periodogram analyses for web-building behavior under DD. Indicated are the periods of web-building behavior (τ_{DD}), in hours, that were detected as significant at $\alpha = 0.001$.

	τ_{DD}	
Identity	chi-square	Lomb-Scargle
Ct-web 5	19.6, 26.1, 29.2	16.8, 23.3
Ct-web 7	18.1, 25.8	18, 22.9, 25.9
Ct-web 8	18.5, 23.8, 27.8	18.5, 23.8, 27.8

2011). In all three cases, the short-period mutant animals entrain to LD cycles with a significantly earlier phase angle compared to the wild type (which in all cases is close to 24-h). For example, heterozygous *tau* mutant hamsters, with a free-running period of 22 h under DD, exhibit activity onsets that are about 4 h early in 24-h LD cycles (Ralph & Menaker 1988). The 19-h Drosophila *per*^{S} mutant has an evening activity peak that occurs about 3 h earlier than normal (Hamblen-Coyle et al. 1992). The correlation between short circadian period and early phasing during entrainment also has been shown in humans (Duffy et al. 2001; Eastman et al. 2015). In fact, advanced sleep-phase syndrome, a rare disorder characterized by very early sleep onset and offset, has been

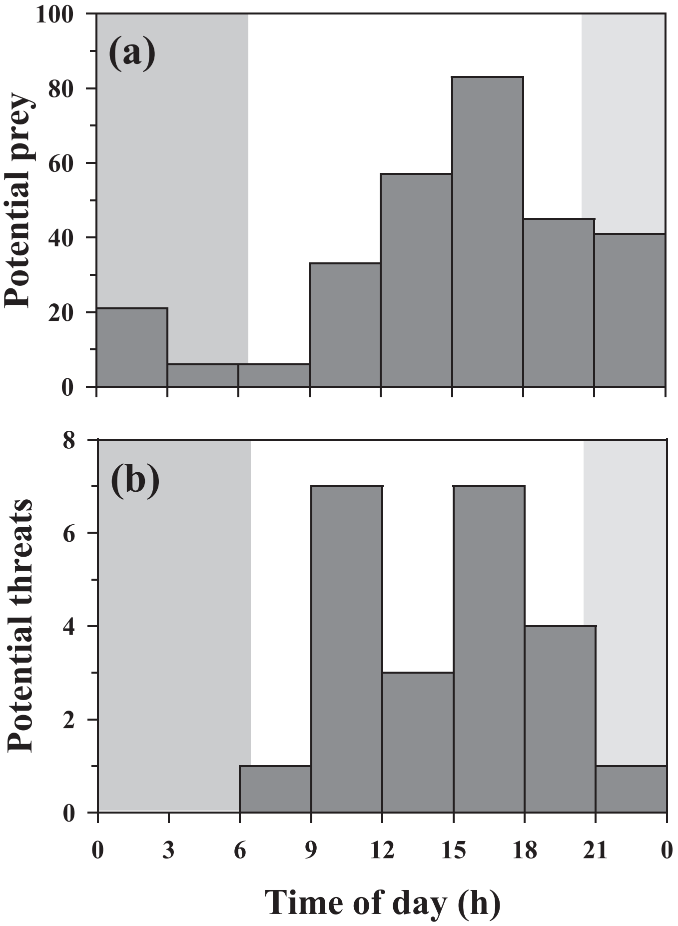

Figure 5.—Data from malaise trap sampling of flying insects in old field *Cyclosa* habitat. Traps were emptied every 3 hours for three consecutive days. Data shown are the combined captures for given time intervals including (a) potential *Cyclosa* prey non-wasp flying insects between 1–15 mm long, and, (b) potential *Cyclosa* threats (wasps). Transitions between shaded and light areas of the graph indicate the time of sunrise and sunset.

attributed to abnormally short-period circadian clocks (Jones et al. 1999). Future experiments are planned to test whether there is a correlation, using a large number of individuals, between the short-period circadian oscillator in *C. turbinata* and phase angle of locomotor and web-building behavior.

Does the short-period clock in *C. turbinata* provide any selective advantage? In most scenarios, one might expect that an extremely short circadian period would be selected against. For example, recent studies suggest, in organisms as diverse as mice (Spoelstra et al. 2016) and cyanobacteria (Woelfle et al. 2004), that natural selection eliminates genotypes with endogenous circadian periods that do not “resonate” with the natural 24-h light/dark cycle. Stringent evidence for adaptiveness of circadian clocks, however, is relatively rare (Johnson 2005). With respect to *C. turbinata*, direct confirmation of adaptive significance may require field experiments in which the period of the clock is manipulated, such that the phase relationship between web-building and the natural day:night cycle is advanced or delayed. For example, deuterium oxide in the drinking water is known to slow the free-running period of the clock in rodents (Suter & Rawson 1968) and cockroaches (Caldarola & Pittendrigh 1974). One might predict that such period lengthening in *C. turbinata* would result in a delay in web-building behavior, causing it to occur later in the scotophase, perhaps intruding into daylight hours. A possible consequence of such phase-shifting of web-building behavior would include greater predation by visual hunters.

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