The present data show uncoupling of leading- and lagging-strand synthesis, implying the continuation of fork opening despite a block in one strand. Uncoupling of simultaneous strand synthesis may occur without disruption of the dimeric Pol III core assembly. When the lesion resides in the lagging strand, a new priming event may enable lagging-strand synthesis to continue, generating a gapped plasmid and a complete double-stranded plasmid molecule from lagging and leading strands, respectively. When the block resides in the leading strand, the lagging-strand Pol III core gets ahead of the leading-strand core, generating a complete double-stranded plasmid from the lagging strand. A partially double-stranded molecule with a single-stranded region (~1 kb) extending from the lesion site to the end of the plasmid is formed from the leading strand. Plasmids of larger size will be required to determine how far DnaB helicase can travel before the whole fork stops. In both orientations, TLS can repair the partially replicated molecule with similar efficiency and a 50-min delay. This delay strongly depends on the chemical nature of the blocking lesion (*8*). Alternatively, the partially replicated intermediates may be processed by regressed fork formation (*10*–*13*). In *E. coli* and in yeast, genetic data have indicated the bypass of specific lesions to require multiple polymerase switches and specific combinations of TLS polymerases (*7*, *14*–*21*). The strategy implemented here will be useful to unravel the complex biochemistry of various TLS pathways in vivo, thus providing a powerful complement to in vitro approaches.

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Illuminating the Circadian Clock in Monarch Butterfly Migration

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Migratory monarch butterflies use a time-compensated Sun compass to navigate to their overwintering grounds in Mexico. Here, we report that constant light, which disrupts circadian clock function at both the behavioral and molecular levels in monarchs, also disrupts the time-compensated component of flight navigation. We further show that ultraviolet light is important for flight navigation but is not required for photic entrainment of circadian rhythms. Tracing these distinct light-input pathways into the brain should aid our understanding of the clock-compass mechanisms necessary for successful migration.

In eastern North America, monarch butterflies (*Danaus plexippus*) undertake a remarkable migration every fall, traveling up to 3600 km to reach their overwintering grounds in central Mexico (*1*). Migrating monarchs use a time-compensated Sun compass to navigate (*2*, *3*). It is not known, however, how the circadian clock interacts with the Sun compass, which enables migrants to maintain a southwesterly flight bearing as the Sun moves across the sky each day.

To investigate the role of the circadian clock in monarch navigation, we first evaluated the basic properties of the monarch circadian system by examining the time of day of adult emergence (eclosion) from the chrysalis (*4*), because eclosion is a reliable marker of circadian function in other insects (*5*). Under laboratory light:dark conditions, adult monarch eclosion was restricted to the early portion of the light period (Fig. 1A, upper panel), as expected from field observations (*6*). A 6-hour shift of the light:dark cycle during adult development caused a corresponding shift in the average timing of eclosion (*7*). The eclosion rhythm persisted in constant darkness, with adults emerging at the correct times as though they had remained in a light:dark cycle (Fig. 1A, middle panel). These results indicate that the monarch eclosion rhythm is controlled by a light-entrained circadian clock. Constant light disrupted the timing of eclosion (Fig. 1A, lower panel), as occurs in other insects (*8*, *9*), demonstrating that constant light provides a means of disrupting the monarch clock.

To monitor the molecular clock after eclosion, we cloned the monarch *period* (*per*) cDNA (GenBank accession no. AY237279) (*4*), because *per* is an essential component of the circadian clock of *Drosophila* (*8*), and *per* RNA levels oscillate in flies and silkmoths (*8*, *9*). Real-time polymerase chain reactions (PCRs) of *per* RNA levels from the heads of the recently emerged monarchs in the eclosion study were examined 24 hours later in the three lighting conditions (*4*). A robust rhythm in *per* RNA levels was detected under the light:dark condition, with high levels during the night and low levels during the day ($P = 0.01$, one-way analysis of variance) (Fig. 1B). The rhythm persisted in constant darkness ($P < 0.01$), with some dampening in amplitude and an apparent advance in timing (Fig. 1B). In the constant light group, the *per* rhythm was severely blunted (Fig. 1B), with RNA levels at constant low daytime values ($P > 0.05$). Constant light thus disrupts the underlying clockwork mechanism, leading to the disruption of output rhythms (such as the timing of adult eclosion).

To study the role of the circadian clock in migratory flight behavior, we used the Mouritsen-Frost flight simulator (*3*, *4*, *10*). We first examined tethered migrants housed in the laboratory under two 12-hours-light: 12-hours-dark lighting conditions, which dif-

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fered in phase from each other by 6 hours. When entrained to a light:dark condition that was close to the fall outdoor lighting cycle [lights on from 0700 to 1900 hours, eastern standard time(EST)], migrants exposed to the outdoor Sun oriented significantly to the southwest, toward Mexico, with a mean vector (α) of 233° ($n = 14$, $r = 0.82$, $P < 0.001$) (Fig. 2, upper panel). Migrants entrained to the advanced light:dark cycle (lights on from 0100 to 1300 hours) also oriented significantly, but to the southeast, with α of 118° ($n = 18$, $r = 0.53$, $P < 0.01$) (Fig. 2, middle panel). The direction and magnitude of the orientation difference between the two groups (a counterclockwise shift of 115°; $P < 0.001$) are those expected for a time-compensated Sun compass that has been advanced by 6 hours (*3*, *11*).

Constant light was used to examine the effects of circadian clock disruption on time-compensated flight orientation. Migrants previously housed under the two light:dark cycles were placed in constant light and evaluated 5 days later. The flight behavior of individuals was still directional in the constant light-exposed migrants. But the orientation direction did not differ between the two light:dark groups of origin ($n = 7$ in each group; $P > 0.05$), indicating loss of circadian control. Instead, the combined analysis of the two groups showed highly synchronized flight orientation, with the migrants flying toward the Sun ($\alpha = 161°$, $n = 14$, $r = 0.73$, $P < 0.001$; Fig. 2, lower panel) (*12*). These results show that the circadian clock is necessary for time-compensated navigation. The residual orientation in the butterflies exposed to constant light may be a positive phototactic response to the Sun itself.

We next investigated whether monarch navigational and circadian behaviors have differential light requirements. We first examined whether migrant monarchs use ultraviolet (UV) light for navigation, given that UV light is used for the navigational activities of other insects (*13*). This was evaluated in migrants that were placed in the flight simulator outdoors under sunny skies. Once video monitoring indicated that a migrant had initiated continuous flight for at least 1 min, the simulator was covered with a UV-interference filter (which blocked light at wavelengths <394 nm) (*14*). All of the 13 butterflies that had initiated flight stopped flying completely or flew only intermittently when the filter was applied. Once the filter was removed, 85% (11 out of 13) of the individuals quickly reinitiated continuous directional flight over the next 5-min period of study. Nine of the butterflies that reinitiated directional flight were migrants that had been housed in outdoor lighting conditions and had a mean flight vector on recovery that was similar to that expected for migrants heading to Mexico ($\alpha = 212°$, $n = 9$, $r = 0.83$, $P < 0.001$) (Fig. 3A) (*15*).The two remaining animals that flew directionally after removal of the UV block were from the constant-light-exposed group. Each of those butterflies flew directionally only when the UV-interference filter was removed, suggesting that they were indeed orienting toward the Sun (Fig. 3B).

To further examine the importance of UV light for monarch flight behavior, four migrants housed under diurnal lighting were examined indoors where a mercury arc lamp functioned as the light source (*4*). The purpose of this experiment was to confirm the importance of UV light for monarch flight in an experimental situation in which we could use a UV-pass filter (which blocked light at wavelengths from 428 to 500 nm), in addition to the UV-interference filter. Each animal that we tested continued to fly toward the artificial light filtered through a UV-pass filter, but each also stopped flying when the UV-interference filter was used (Fig. 3C and fig. S1). The combined results of the outdoor and indoor experiments show that a UV photoreceptor is required for sustained flight (*16*).

In contrast to the importance of UV light for navigation, UV light was not required for photic entrainment of the monarch circadian clock.

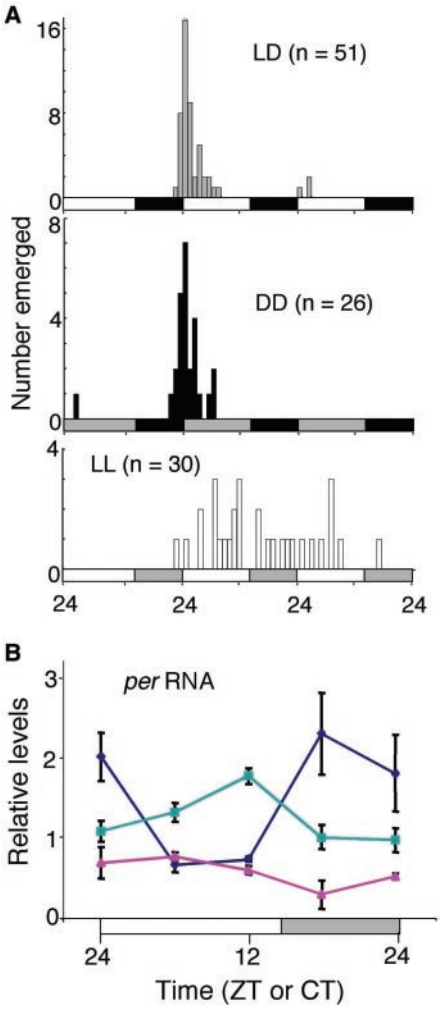

Fig. 1. (**A**) Patterns of adult eclosion in 14-hours-light:10-hours-dark (LD) conditions, constant dark (DD) conditions, and constant light (LL) conditions, monitored at 1-hour intervals. Eclosion occurred on the third day under DD conditions and on the third and fourth days under LL conditions. Open bars, light; black bars, dark; gray bars, subjective day (for DD) or night (for LL). (**B**) Patterns of *per* RNA levels from the adult monarchs who emerged in (A) and were studied 24 hours later in their respective lighting conditions (blue, LD; green, DD; magenta, LL). Relative levels were normalized to the corresponding *rp49* levels. Each value is the mean ±SEM from four heads. Similar *per* RNA patterns were seen with a second set of animals housed under the three lighting conditions.

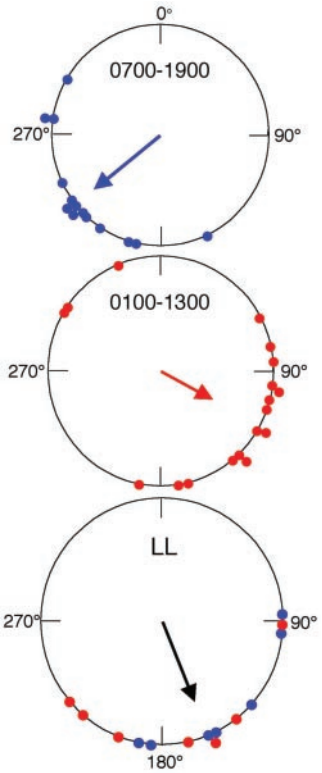

Fig. 2. Flight orientation of migrants housed under different lighting conditions. The large circle represents the 360° of possible directions (0° = north); small solid circles on the perimeter represent the flight orientation of individual butterflies. The upper circle shows migrant butterflies housed in a light:dark cycle with lights on from 0700 to 1900 hours. The middle circle shows migrants housed in a light:dark cycle with lights on from 0100 to 1300 hours. The lower circle shows migrants from 0700 to 1900 (blue) or 0100 to 1300 (red) lighting cycles that were subjected to 5 days of constant light before being studied outdoors in a flight simulator. The arrow indicates the mean vector, and the length of the arrow represents the strength (*r* value) (*4*). Video monitoring showed that all included butterflies were flying continuously during the study period (between 10 and 20 min, mean flight time 14.4 min). Moreover, the construction of virtual flight paths from the computer records [as in (3)] showed that butterfly flight was directional (fig. S2).

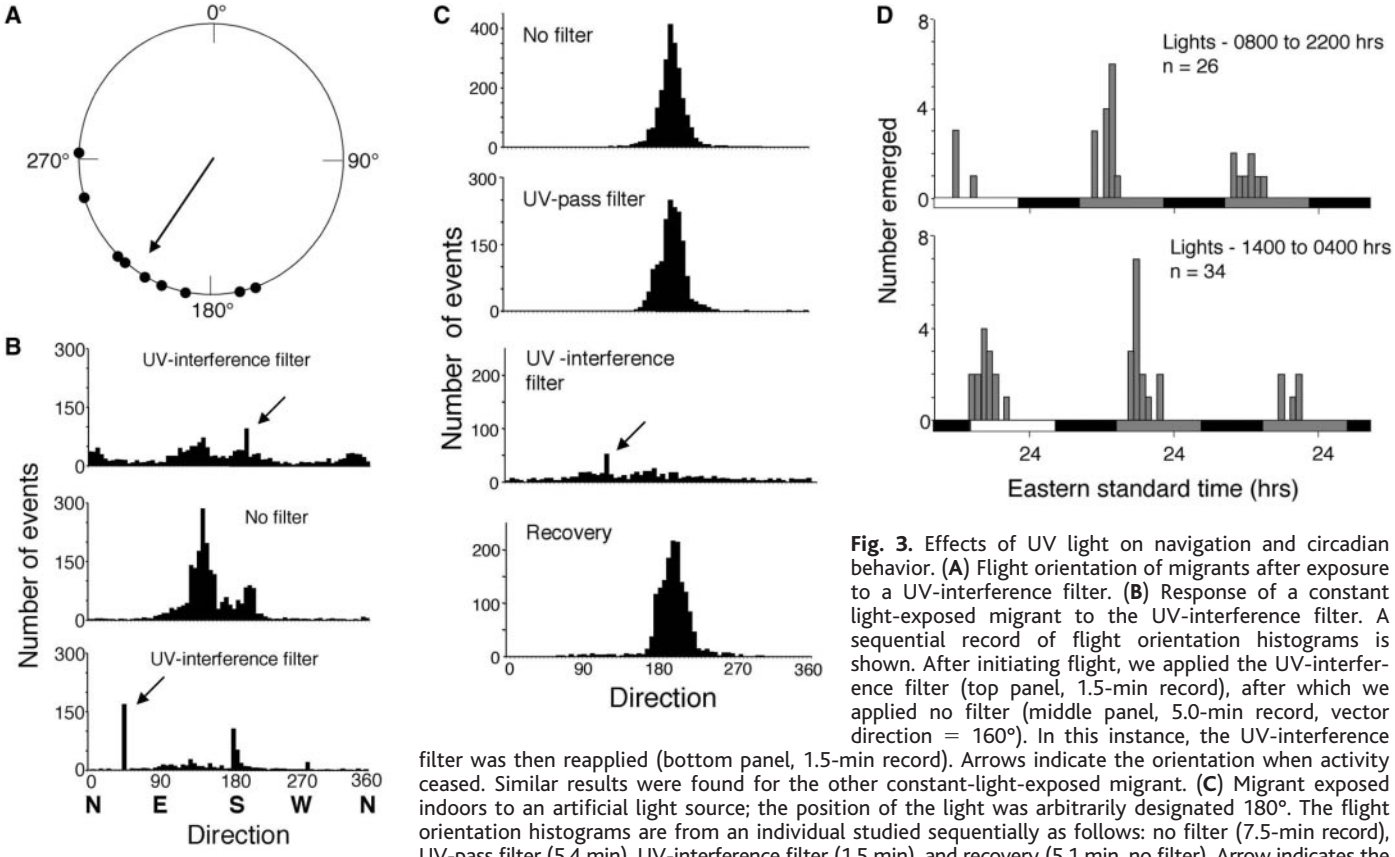

Fig. 3. Effects of UV light on navigation and circadian behavior. (**A**) Flight orientation of migrants after exposure to a UV-interference filter. (**B**) Response of a constant light-exposed migrant to the UV-interference filter. A sequential record of flight orientation histograms is shown. After initiating flight, we applied the UV-interference filter (top panel, 1.5-min record), after which we applied no filter (middle panel, 5.0-min record, vector direction = 160°). In this instance, the UV-interference filter was then reapplied (bottom panel, 1.5-min record). Arrows indicate the orientation when activity ceased. Similar results were found for the other constant-light-exposed migrant. (**C**) Migrant exposed indoors to an artificial light source; the position of the light was arbitrarily designated 180°. The flight orientation histograms are from an individual studied sequentially as follows: no filter (7.5-min record), UV-pass filter (5.4 min), UV-interference filter (1.5 min), and recovery (5.1 min, no filter). Arrow indicates the orientation when activity ceased. Similar results were obtained from the three other migrants studied (shown in fig. S1). (**D**) Patterns of adult eclosion in two 14-hours-light:10-hours-dark cycles that lacked UV light and differed in phase by 6 hours. The data for both groups are plotted relative to EST. Open bars, light; black bars, dark; gray bars, subjective day.

This was shown by analyzing the circadian timing of adult eclosion behavior. Newly formed pupae were exposed to one of two light:dark cycles, which differed in phase from each other by 6 hours, in which all light was filtered through the UV-interference filter. Circadian entrainment to the two lighting cycles that lacked UV light was evaluated by placing the pupae in constant darkness as the animals became mature enough to eclose. For each group, animals eclosing in constant darkness did so during the early portion of what would have been the light period of the lighting cycles (Fig. 3D). The mean peak times of eclosion, as monitored for 2 days in constant darkness, differed between the two groups by 5.5 hours (as compared with the 6.0-hour shift of the lighting cycles) (Fig. 3D). The data indicate that the circadian clock was properly entrained by light of a wavelength >394 nm (*17*).

Our results provide insights into time-compensated Sun compass navigation in migratory monarch butterflies. The necessity for circadian control for the time-compensation component of monarch navigation shows that a functioning clock is essential for successful migration. Moreover, the molecular gears of the monarch circadian clock (such as *per*) are likely the first identified genetic components underlying migratory behavior. Examination of the lighting requirements for time-compensated Sun compass navigation suggests that there are distinct light-input pathways for the stimulation of oriented flight behavior (UV-dependent) and entrainment of the circadian clock (UV-independent). Tracing these pathways into the brain should aid our understanding of the clock-compass interface and further illuminate the mechanisms of monarch butterfly migration.

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10. Outdoor experiments were performed under sunny conditions in Worcester, MA (latitude 42°16′N, longitude 71°49′W) in an outdoor arena between 0900 to 1300 hours EST from 21 September through 21 October 2002.
11. The Sun's azimuth varied from 16° to 22° per hour during the time of day of study, which would result in a 6-hour shift of between 96° and 132°.
12. The azimuth of the Sun during the constant-light study was between 136° and 160°.
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14. The UV-interference filter was a long-wavelength pass filter with a photopic luminous transmission of 87% (E400 from Gentex, Carbondale, PA). Transmission values were as follows: 5% ≥ 394 nm, 50% = 398 ± 6 nm, and 80% ≤ 415 nm.
15. We found no significant difference in flight orientations computed from the first 5 min of continuous flight as compared with those from 10- to 20-min flight records.
16. Based on studies in other navigating insects (*13*, *18*), it is likely that the polarization pattern of skylight is involved in monarch navigation. In fact, the dorsal rim of the monarch butterfly eye contains some ommatidia with orthogonal microvilli (*19*), an anatomical hallmark of polarized skylight detection (*13*).
17. Based on studies in *Drosophila*, the major monarch circadian photoreceptor is likely to be a blue light–sensing cryptochrome (*20*), which has been cloned (*21*).
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