SUPPLEMENTAL INFORMATION

Materials and Methods

Animals: Monarch chrysalises were purchased from commercial sources. Within 72 hrs after pupation, they were transferred to environmental compartments maintained at 21° - 23° C. The exact time of adult emergence was monitored by a video camera connected to a video recorder.

Diapausing monarch butterflies migrating through Western Massachusetts were captured between September 15 and October 10, 2002. For most flight experiments, butterflies were placed in glassine envelopes and housed in environmental compartments in the laboratory at 18° C and 60% humidity. Animals were fed every 3rd day. The daily lighting cycle in the compartments was controlled automatically with white light provided by 20W cool white fluorescent tubes. The dark portion of the lighting cycle was composed of dim red light provided by 20W litho number 2 fluorescent tubes (Chemical Products Co.); these red lights also remained on during constant darkness. Migrants were housed for at least 5 days under any of the lighting conditions before study in the flight simulator.

Flight behavior: Flight behavior of tethered butterflies was monitored using the Mouritsen-Frost flight simulator, as described (1). Flight direction was recorded by computer running USB1 Explorer (US Digital) configured to record the direction of flight every 200 ms. Flight was monitored visually through a small hole in the bottom of the simulator by an externally mounted surveillance camera.

For the butterflies studied in outdoor experiments shown in Figure 2, the mean flight time was 14.4 min. In general, butterflies were taken out of the simulator after 15 min so that others could be studied. One animal not shown or used in the analysis flew continuously in the simulator for 4.5 hrs. Individuals were not re-used, except for 3 animals reported in Fig. 3a that were used two days later (for data shown in Fig. S1). Total number of migrants used in the reported studies was 60.

For each group shown, the angle and length of the mean flight vector and its significance was calculated using the Rayleigh test, and comparisons between groups were done using the Mardia-Watson-Wheeler test for two samples (2).

Indoor experiments were conducted in a windowless room with the room lights turned off. The artificial light was a 100 watt mercury arc lamp (Olympus Optical Co.) positioned

above the simulator at a 70˚ angle from horizontal which delivered 9.2 mW/cm^2 light to the monarch head; 32% of the lamp output was from 260 to 400 nm. The front of the light source (diameter 4.5 cm) was 40.6 cm from the butterfly head. The UV-pass filter (VIS-NIR short pass filter) has a transmittance range from 240 – 400 nm (>85% transmittance) and a blocking range from 428-500 nm (Model 57396, Oriel Instruments). Light measurement at the head was 8.0 mW/cm^2 with the UV-interference filter and 7.3 mW/cm^2 with the UV-pass filter.

Cloning monarch *period* cDNA: Degenerate PCR primers based on lepidopteran *period* sequences (3) were used to clone a 400 bp fragment of the monarch *period* cDNA. 5' and 3' rapid amplification of cDNA ends was then used to clone the entire coding region.

Real-time PCR: DNase-treated total RNA was reverse transcribed using Superscript II RNase H$^-$ Reverse Transcriptase (Invitrogen). A portion of the reaction was subjected to quantitative PCR using the Platinum qPCR Supermix-UDG (Invitrogen) and the ABI Prism 7000 Sequence Detection System. PCR primers (forward, reverse, and probe): *per* forward 5'-GGCTGTCACGTATGCACCAA –3'; *per* reverse 5'-CCACGACGGATCAACCTTTT-3'; *per* probe 5'-6FAM-AAGAGCCAGGCACAAC-MGB-3'; *rp49* forward 5'-CCGGAAGGTGTTAGTCCACAAC-3' *rp49* reverse 5'-CGGCGCAGTACTTCCTATTCTG-3'; and *rp49* probe 5'-6FAM-TGAGCTGGAAATCC-MGB-3'.

References:

1. H. Mouritsen, B. J. Frost, *Proc. Natl. Acad. Sci. USA* **99**, 10162-10166 (2001).

2. E. Batschelet, *Circular Statistics in Biology*, New York: Academic Press (1981).

3. J. C. Regier *et al., Mol. Biol. Evol.* **9**, 1172-82 (1998).

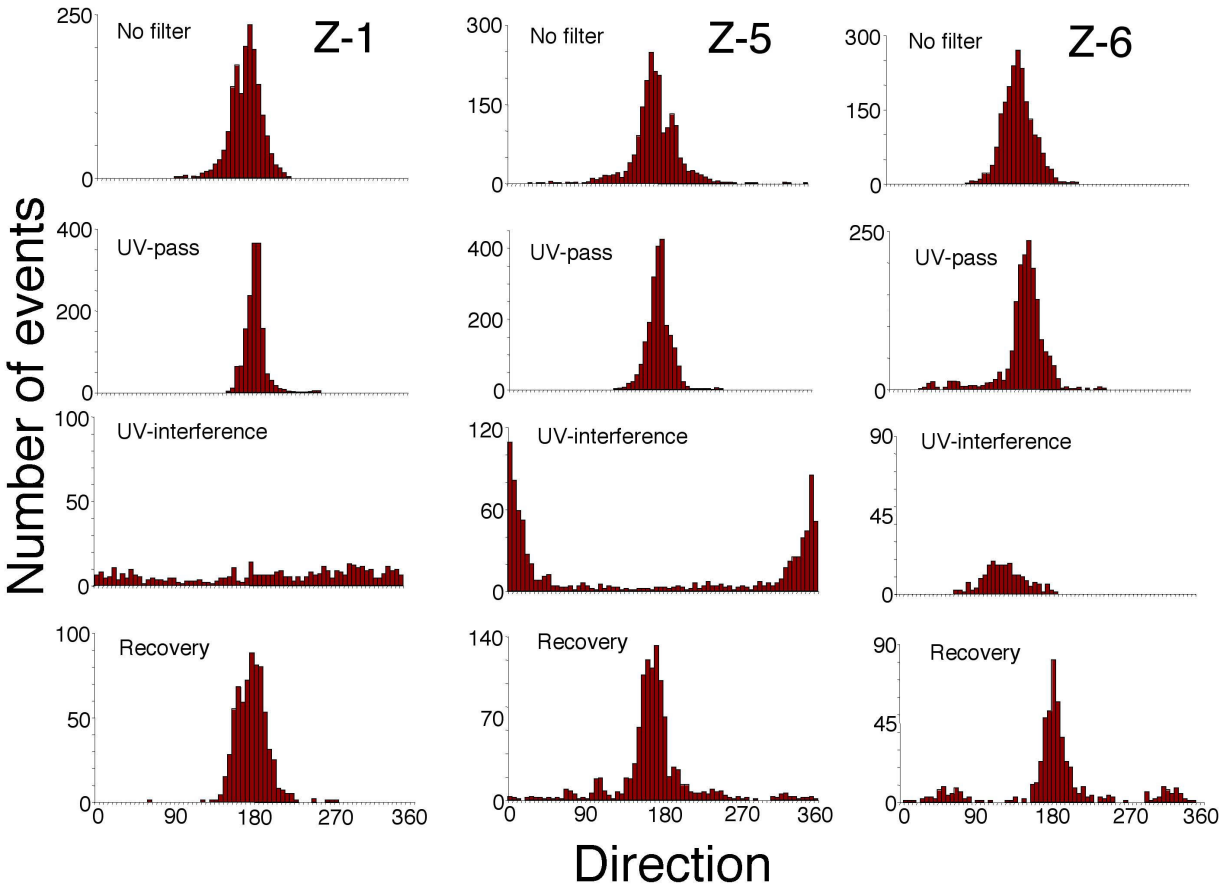

Figure S1: Effect of UV light on indoor flight behavior. Migrants were exposed indoors to an artificial light source; the position of the light was designated 180˚. The flight orientation histograms are from three individuals, each studied sequentially: no filter, UV-pass filter, UV-interference filter, and recovery (no filter). Video monitoring showed that all animals stopped flying within 15 sec of applying the UV filter. This includes Z-5, who was struggling and not flying during the entire time the UV-interference filter was in place (the "events" recorded represent thrashing legs and erratic wing flapping), based on observation through the surveillance camera.

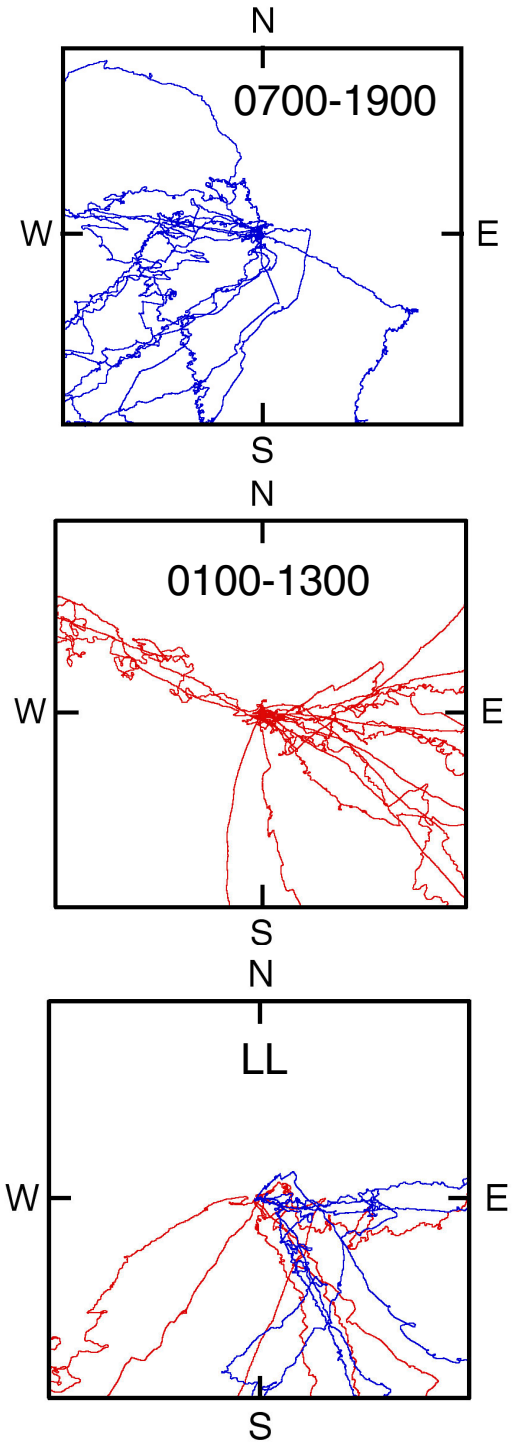

Figure S2: Virtual flight paths of the data depicted in Figure 2. These were constructed by starting in center of the box and plotting each direction interval consecutively as one unit length (see Ref. 1). The data have been normalized so that the entire flight path of each butterfly is shown.