Supplemental Data: The Two CRYs of the Butterfly

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Supplemental Experimental Procedures:

Cloning and Sequence Analysis

cDNA fragments were cloned by either prime-specific or degenerate PCR. cDNA templates for PCR were prepared from RNA purified from monarch butterfly brains or mosquito heads. The ends of the coding regions were obtained by rapid amplification of cDNA ends (RACE; Clontech kits). Complete open reading frames were obtained by Pfu Turbo (Stratagene) PCR from cDNA. Clones were sequenced at core facilities at UMass Medical School. Sequence analysis was facilitated by software from the Genetics Computing Group (GCG) and the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/BLAST/). GenBank accession numbers for the full-length coding regions are: dpCLK, AY364477; dpCYC, AY364478; dpCRY2, DQ184682; agCRY1, DQ219482; and agCRY2, DQ219483.

Insect cell culture, Transfections, and Transcription Assays

S2 cells were maintained at 25°C in Schneider's *Drosophila* medium (Invitrogen) with 9% heat-inactivated fetal bovine serum (Invitrogen). The reporter was generated by subcloning a tandem repeat of an E box element from the monarch *per* gene promoter into a luciferase reporter vector containing the *hsp 70* promoter [S1]. S2 cell expression constructs were generated by subcloning cDNAs into the pAc5.1V5/HisA vector (Invitrogen). Transient transfections and transcriptional assays were performed as previously described [S1].

Western blots

Western blotting was performed as described previously [S2]. The V5 antibody used for western blots was a monoclonal mouse anti-V5 IgG purchased from Invitrogen.

Supplemental Figure S1

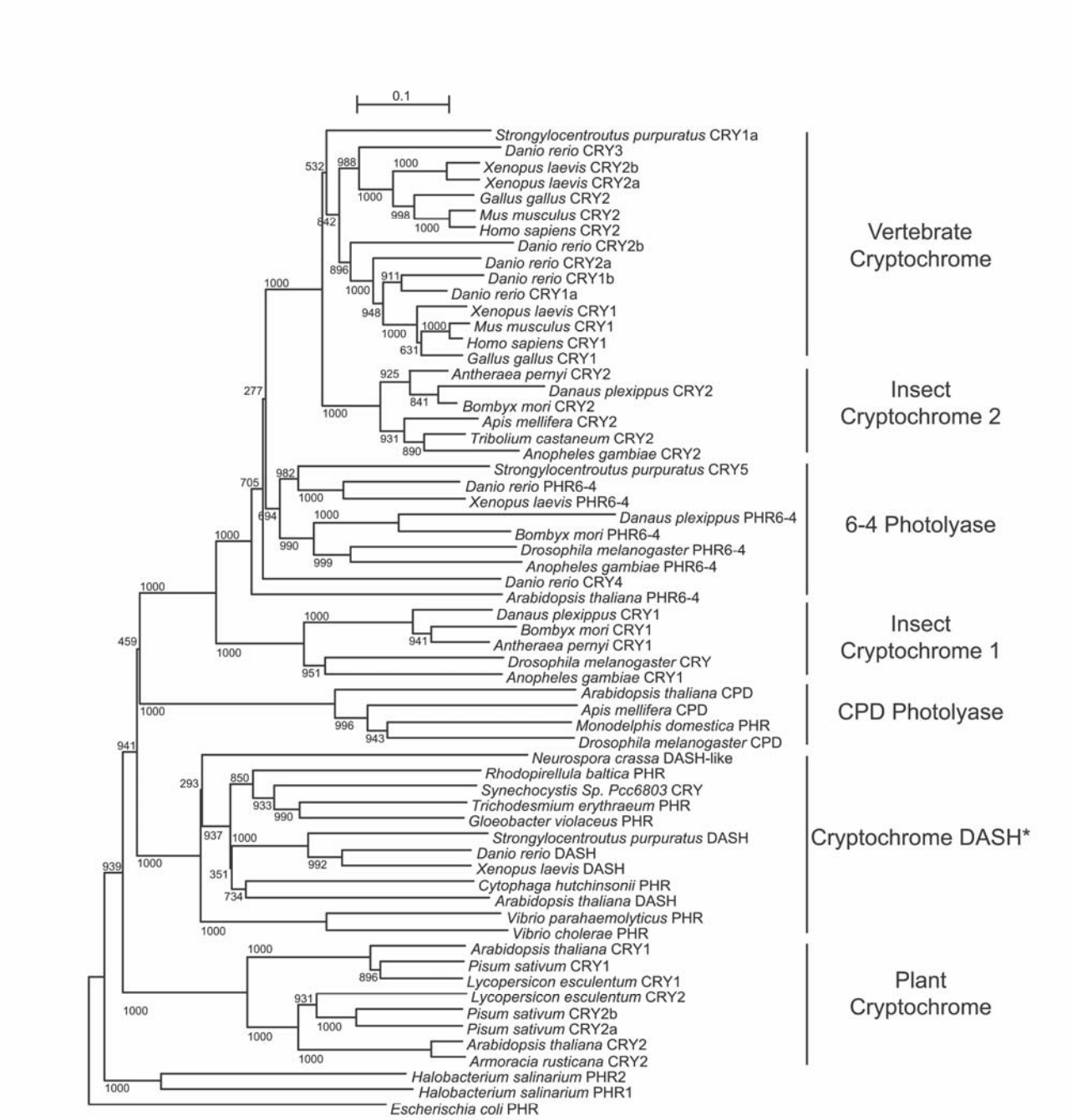

Figure S1. Phylogenetic tree of members of the photolyase/cryptochrome families. Bootstrap values (of 1000 replicates) are indicated on horizontal branches. CLUSTAL X

program was used to analyze the following amino acid sequences (GenBank™ accession number unless otherwise indicated): *Anopheles gambiae* CRY1 (DQ219482), CRY2 (DQ219483), PHR6-4 (EAA10141); *Apis mellifera* CPD (XP_624004), CRY2 (XP_393680); *Antheraea pernyi* CRY1 (AAK11644); *Arabidopsis thaliana* CPD (CAA67683), CRY1 (Q43125), CRY2 (Q96524), CRY-DASH (BAC65244), PHR6-4 (NP_566520); *Bombyx mori* CRY2 (NRPG1215 and brP-1009, EST database, Silkbase, Japan, http://papilio.ab.a.u-tokyo.ac.jp/silkbase/index.html), CRY1, PHR6-4 (Scaffold008995, Scaffold004296, genome project, Beijing Genomic Institute, China, http://silkworm.genomics.org.cn/index.jsp *Science* 306:1937, 2004); *Cytophaga hutchinsonii* PHR (ZP_00117704); *Danaus plexippus* CRY1 (AY860425), CRY2 (DQ184682), PHR6-4 (EST library, unpublished); *Danio rerio* CRY1a (BAA96846), CRY1b (BAA96847), CRY2a (BAA96848), CRY2b (BAA96849), CRY3 (BAA96850), CRY4 (BAA96851), CRY-DASH (NP_991249), PHR6-4 (NP_571863); *Drosophila melanogaster* CPD (BAA05042), CRY (AAC83828), PHR6-4 (BAA12067); *Escherichia coli* PHR (P00914); *Gallus gallus* CRY1 (NP_989576), CRY2 (NP_989575); *Gloeobacter violaceus* PHR (NP_923781); *Homo sapiens* CRY1 (NP_004066), CRY2 (NP066940); *Halobacterium salinarum* PHR1 (NP_280501), PHR2 (NP280191); *Lycopersicon esculentum* CRY1 (AAF72555), CRY2 (AAF72556); *Mus musculus* CRY1 (NP_031797), CRY2 (AAD46561); *Monodelphis domestica* PHR (S50083); *Neurospora crassa* CRY-DASH like hypothetical protein (EAA36486); *Pisum sativum* CRY1 (AAS79662), CRY2a (AAS79665), CRY2b (AAS79667); *Rhodopirellula baltica* PHR (CAD77347); *Strongylocentroutus purpuratus* CRY1a (XP_785873), CRY5 (XP_788938), CRY-DASH (XP_783613); *Synechocystis Sp. Pcc6803* CRY (1NP7A); *Tribolium castaneum* CRY2 (Contig 3142, Genome Project, Baylor College of Medicine, http://www.hgsc.bcm.tmc.edu/projects/tribolium/); *Trichodesmium erythraeum* PHR (ZP_00071643); *Vibrio cholerae* PHR (B82155); *Vibrio parahaemolyticus* PHR (BAC61546); *Xenopus laevis* CRY1 (AAK94665), CRY2a (AAK94666), CRY2b (AAK94667), CRY-DASH (AB120760), PHR6-4 (BA97126). *E. coli* PHR was used as the outgroup. (*Members of CRY-DASH protein family are defined in Reference S3 based on sequence similarity.)

Supplemental References:

S1. Chang, D.C., and Reppert, S.M. (2003). A novel C-terminal domain of Drosophila PERIOD inhibits dCLOCK:CYCLE-mediated transcription. Curr. Biol. *13*, 758-762.

S2. Lee, C., Etchegaray, J.-P., Cagampang, F.R.A., Loudon, A.S.I., and Reppert, S.M. (2001). Posttranslational mechanisms regulate the mammalian circadian clock. Cell *107*, 855-867.

S3. Lin C., and Todo, T. (2005). The cryptochromes. Genome Biology *6*, 220.1-220.9.