Figure S1: *pyrexia* is required for synchronization to 16°C : 20°C temperature cycles in constant light. (a) *pyx* mutant flies of the indicated genotypes were analyzed in LD, followed by two phase delayed 16°C : 20°C temperature cycles (TC) in constant light (LL) as...
described as in the legend to figure 1. (b) Plotting of the EI with error bars indicating SEM. Statistical significance was assessed by one-way ANOVA followed by post hoc test (* p values < 0.05, **** values < 0.0001). All pyx alleles and combinations are significantly different from Canton S.
Figure S2: *pyrexia* is required for synchronization to 16°C : 20°C temperature cycles in constant darkness. (a) *pyx* mutant flies of the indicated genotypes were analyzed in LD, followed by two phase delayed 16°C : 20°C temperature cycles (TC) in constant darkness (DD) as described as in the legends to figures 1 and 2. (b, c) Plotting of the EI for the last 3 days of TC2 (b) and the 1st three days of constant conditions (c) with error bars indicating
SEM. Statistical significance was assessed by one-way ANOVA followed by Fisher’s LSD post hoc test (* p values < 0.05, ** p values < 0.01, ns: not significant). All pyx alleles and combinations are significantly different from Canton S, except $pyx^{dp9}$ in free running conditions (due to high SEM).
Figure S3:

(a) CantonS

(b) pyx³

(c) pyx³/pyx⁹⁹

(d) pyxGeV pyx³
**Figure S3:** *pyrexia* locks free-running phase with synchronized peak activity in 16°C : 20°C temperature cycles in constant darkness. (a-d) Control and *pyx* mutant flies of the indicated genotypes were exposed to three (left panels; ‘shift flies’) or only one (right panels; ‘constant temperature control flies’) 16°C : 20°C temperature cycles (TC) before being released to constant 20°C. TC1 was in-phase with the previous LD cycle (not shown), to make sure that all flies are synchronized before being released to constant conditions. For plotting and figure labelling, see legend to figure 1. Comparison of peak activity phases during the final days of the experiment between the shift and constant temperature control groups shows that the Canton S and *pyx*Ge *pyx*³ flies adopt almost opposite phases. In contrast, the *pyx* mutant flies show more similar phases between the two groups indicating that synchronization to TC is impaired.
Figure S4:

(a)

LD 25°C
TC1 20°C-25°C
TC2 20°C-25°C
DD 20°C

(b)

LD 25°C
TC1 20°C-25°C
TC2 20°C-25°C

CantonS
pyx3
pyx3/pyx3
pyxGe pyx3
**Figure S4:** pyrexia is not required for synchronization to 20°C : 25°C temperature cycles. pyx mutant flies of the indicated genotypes were analyzed in LD, followed by two phase delayed 20°C : 25°C temperature cycles (TC) in DD (a) and LL (b) exactly as described in the legends to figures 1 and 2. Low and high temperatures are indicated in light and dark orange, respectively. Note that all genotypes show synchronized activity peaks during this temperature interval.