signalling is important for timely SIN resetting (Figure 2). Globally, their model proposes that etd1p is required for SIN activation, but that the active SIN in its turn inactivates etd1p, perhaps by promoting its degradation. During anaphase, the balance initially favours etd1p, and it promotes an increase in SIN activity. At the time of septum formation when *etd1* expression declines [6], the active SIN gains the upper hand, and etd1p activity declines. As etd1p is required for SIN activity, SIN signalling from the nSPB is auto-extinguishing (Figure 2). The APC/C subunit nuc2p has been implicated in resetting the SIN [12]; it will be of interest to determine whether it is involved in the degradation of etd1p.

The new paper [3] shows that cells expressing elevated levels of GFP–etd1p retain some cdc7p on the nSPB after cleavage, consistent with a delay in resetting the SIN. Filming of dikaryons suggests that SIN asymmetry is required for the differential changes in etd1p level that are observed in the daughter cells. The mechanism whereby closure of the contractile ring is coupled to SIN inactivation remains to be elucidated. This study does not address what establishes the initial SIN asymmetry in anaphase B, though cdk inactivation is clearly important [13,14]. In SIN mutants, the GAP remains asymmetric [15,16], suggesting that the establishment of SIN protein asymmetry is mediated via the GAP, though this remains conjectural.

In summary, this paper [3] sheds new light on SIN regulation and builds upon the earlier proposition [6] that etd1p degradation could be coupled to SIN inactivation, incorporating a role for the mitotic asymmetry of the SIN proteins. Understanding how the mutual regulation of etd1p and the SIN works will be of great interest. We speculate that if etd1p activates the SIN, then perhaps the contractile ring-dependent medial pool of GFP–etd1p observed in early mitosis [6] contributes to the SIN's early mitotic activity in contractile ring formation.

Finally, we note that the observation that the progeny of a single division differ with regard to their treatment of GFP–etd1p provides another instance of an asymmetric event in the 'symmetric' fission yeast cell, which include the regulation of mating-type switching [17], maturation of spindle pole bodies over two cell cycles [4], and the growth pattern and segregation of cell polarity factors [18]. It will be of interest to determine whether the SPB inherited by a cell affects any other aspects of its biology.

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Circadian Clocks: Evolution in the Shadows

As scientists, we strive for highly controlled conditions. The real world, however, is noisy. Complex networks are a coping mechanism for an erratic environment.

**Martha Merrow*
and Marc F.P.M. Maas**

The field of genetics has elaborated a multitude of partially defined complex networks. An excellent example is the molecular mechanism driving the circadian biological clock (Figure 1). The clock is a fundamental process that permeates biology at all levels, creating a temporal structure that serves to anticipate what is needed by the cell and the organism, and when. Originally characterized as a simple, single feedback loop, the molecular circadian network is presently described as a collection of transcription factors that form interlocked loops [1]. In an attempt to understand the inherent complexity of the circadian clock, a group of systems biologists, as reported in this issue of *Current Biology*, has applied (relatively) unbiased iterative modeling to the

problem of network evolution [2]. For their simulations, Troein *et al.* [2] fed the model alternatively with either smooth, square-wave or noisy (i.e., realistic) information to evoke daily light cycles. They asked what kinds of networks would arise and they discovered that features of the light environment that derive from real weather as well as day length promote complexity.

The molecular clock has been largely defined using free-running, self-sustained oscillation as an assay despite the fact that the clock is always entrained or synchronized in nature to cycling conditions. Throughout evolution, the clock has been shaped by zeitgeber (from the German for 'time-giver') cycles that are repetitive with respect to day and season. Light is generally considered the most important zeitgeber [3], and it is certainly the best characterized for its effects on circadian clocks, albeit most often in the form of a discrete light pulse.

An important aspect of the light environment, however, is day length, which regulates, for example, reproduction via the circadian clock [4]. Photoperiod can be predicted to the minute for centuries past and to come; however, over the day, the light environment can change from one minute to the next (for example, via cloud cover; Figure 2). At least for plants and animals, an indication of the mechanism of biological photoperiodism is the expression of key clock genes around dawn and dusk, irrespective of the day length [5,6]. The induction of an RNA species at dawn could be caused by a simple light induction, but for gene expression to anticipate dusk in different photoperiods demands a sophisticated timing mechanism. The function of the circadian system is to provide a reliable temporal structure (hence the word 'clock') according to photoperiod and despite a light environment that can change from day to day or even within a day. Indeed, earlier modeling experiments have demonstrated that noise can serve a stabilizing function in a network of feedback loops [7].

The experiment that Troien *et al.* [2] performed began with an unspecified network of four genes. Interaction between any two components or even feedback on self was allowed; delays were included as parameters that could be modified, a feature that would allow some *in silico* post-transcriptional regulation, thought to be necessary to achieve a 24 hour oscillation. Any of the genes could be regulated by a light signal, although one component was specified as the dawn component and the other as the dusk one; they had to be expressed in specified time windows relative to the photoperiod. Simulations were run 'under entrainment' in conjunction with various selection procedures (fitness testing and pruning to keep the number of feedbacks somewhat constrained). The resulting networks increased in interconnectedness as the entrainment moved from single photoperiod to multiple photoperiods, but only the combination of various photoperiods together with a realistic, noisy light environment — actually derived from a year of recordings in the Harvard Forest — yielded a highly interconnected network, showing that complexity is an outcome of real weather.

Many interesting implications flow from this work. One of these concerns how light is taken up by the circadian clock. Both the timing and amount of light administered can change the phasing of clock-regulated processes [8]. Hence, understanding how light acts on the clock is as important as understanding the clock mechanism itself. The input pathway is the first step in the process and, although a number of photoreceptor molecules are known for plants, animals and fungi [9–13], signal transduction leading from outer to inner worlds is poorly filled in. For instance, while it was once thought that light acted by acutely inducing clock gene RNAs [14], it was later demonstrated that the RNAs and translation of their proteins are differentially regulated by light in entrainment [15]. Recent work using mice suggests that it is primarily the chronic rather than acute effects of light that are determining entrained phase (when in the day an individual is active) [16]. This observation should change our concepts of entrainment, which have previously been built on the assumption of rapid, discrete phase shifts. Modeling (perhaps even the models derived here) could be used to predict key features of light signaling to the clock network that meet both the demands and constraints of the biological system and the reality of the physical environment.

Second, the modeling suggests that a non-noisy light environment fails to stimulate development of a network that shows a self-sustained, oscillating rhythm in constant conditions. This feature is often considered to be a hallmark of a circadian clock. One problem with equating free running rhythms with a clock is that a failure to find rhythmicity could represent an experimental failure rather than a *bona fide* absence of a biological clock. In support of this line of reasoning is the systematic circadian entrainment that can be demonstrated in some mutants that do not show a robust free running rhythm [17]. The model that Troien *et al.* [2] generated with simple, square wave photoperiods as light cycles manages to show circadian entrainment, as

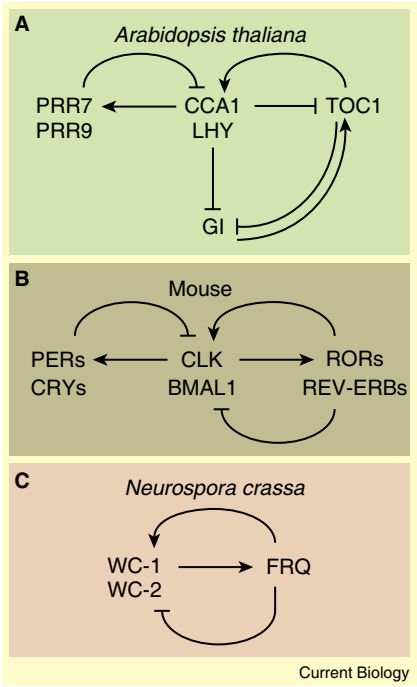

Figure 1. Eukaryotic circadian clock networks.

Eukaryotic circadian clock networks, as defined by genetic studies, form regulatory loops with complex regulation. Some of the best studied clock networks include those controlling circadian rhythms in *Arabidopsis thaliana* (A), mice (B), and the fungus, *Neurospora crassa* (C). Arrows indicate positive effects on expression levels (suggesting transcriptional activation); crossbars indicate negative effects (suggesting transcriptional repression). In addition, each system potentially utilizes multiple light input pathways working at various nodes of the network. Post-translational modifications are common, including phosphorylation, acetylation and sumoylation [18]. Sub-cellular localization of clock proteins is also of key importance [19,20], with rapid shuttling between nucleus and cytoplasm in some cases.

Figure 2. Environmental variability.

In many parts of the world, environmental light can change, practically from one minute to the next, due to cloud cover. The scene here shows pictures taken within several minutes on a day in March 2009 in the Netherlands, illustrating the noisy light environment.

demonstrated by the expression of specified activities at dawn and dusk in different 'seasons', despite failure to show robust free running rhythm. Back in the biological realm, there could be a lot of clocks out there that remain unexplored only for lack of a self-sustained rhythm, despite exhibiting entrainment as a clock should.

Third, one might predict that related organisms evolved in different climates would evolve higher or lower complexity in their clock networks. Weather can be remarkably stable or highly unpredictable depending on proximity to coastlines, latitude, etc. A systematic and comparative investigation of sister species taken from both types of climate should reflect the principles elucidated by the modeling experiment. The model that was selected via the noisy environment is more complex and it is also more successful as selected in the context of the realistic zeitgeber cycle. Thus, experiments should reveal differences in actual clock networks evolved in different sorts of climates. For instance, the period of the free running rhythm at different incubation temperatures or qualities of the entrained phase might be more or less stable, reflecting compensation mechanisms of the metabolic networks to noise. The model also predicts that self-sustained rhythms would be less common in organisms from less noisy environments.

Finally, in the era of systems biology and genetics, the phenomenon of complex regulatory networks, similar to what has been discussed here, is not unusual. The principles that apply to an evolving circadian network might also apply to other networks, such as the cell cycle or developmental pathways. The prediction is that complexity contributes to compensation mechanisms: namely, it contributes robustness against noise from the inner and outer worlds. This generalization might be applied towards understanding functional attributes of (non-clock) networks.

"If you don't like the weather, just wait a few minutes" is a well-known adage to New Englanders. We (the authors) live in the Netherlands, where they say "Kermis in de hel" (it's a carnival in hell), referring to occasionally unpredictable and simultaneously conflicting weather conditions. In much of the world — at least in the temperate zones — the natural zeitgebers are rife with interference due to weather. Complexity in genetic networks will serve to preserve biological function in the face of reality, which is erratic.

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Report

Weather and Seasons Together Demand Complex Biological Clocks

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Summary

The 24-hour rhythms of the circadian clock [1] allow an organism to anticipate daily environmental cycles, giving it a competitive advantage [2, 3]. Although clock components show little protein sequence homology across phyla, multiple feedback loops and light inputs are universal features of clock networks [4, 5]. Why have circadian systems evolved such a complex structure? All biological clocks entrain a set of regulatory genes to the environmental cycle, in order to correctly time the expression of many downstream processes. Thus the question becomes: What aspects of the environment, and of the desired downstream regulation, are demanding the observed complexity? To answer this, we have evolved gene regulatory networks in silico, selecting for networks that correctly predict particular phases of the day under light/dark cycles. Gradually increasing the realism of the environmental cycles, we have tested the networks for the minimal characteristics of clocks observed in nature: oscillation under constant conditions, entrainment to light signals, and the presence of multiple feedback loops and light inputs. Realistic circadian gene networks are found to require a nontrivial combination of conditions, with seasonal differences in photoperiod as a necessary but not sufficient component.

Results and Discussion

Evolving Clock Networks

Starting from randomly connected networks of genes (Figure 1A), we have used a genetic algorithm to create clock networks in which one gene is designated to be expressed just after dawn and another just before dusk. This pattern exemplifies the well-characterized rhythmic profiles of core circadian clock genes, such as *Per1* and *Per2* in the mammalian suprachiasmatic nucleus or *PRR9* and *GI* in *Arabidopsis* [1]. A fitness function measures how well the network times expression of the dawn and dusk genes. Our approach differs from earlier work, which sought specifically for oscillations in constant conditions [6–8]. These works demonstrated that it is possible to evolve simple networks that oscillate and can be entrained to a light/dark cycle. We now use this technique to address the fundamental question of which properties of the environment are required to evolve the complex circadian networks found in nature.

To probe the role of the environmental input, we evolved networks under a range of light conditions. The most basic was alternating 12 hours of light and darkness (LD 12:12), and we extended this in two directions: multiple photoperiods and noise in the timing of the light signal. The former mimics seasonal differences, hypothesized to be important for the emergence of complex clocks [9], whereas the latter represents weather and other stochastic effects on the system. The effects of molecular noise on circadian clocks have been studied extensively [10–14], showing that simple one-loop oscillators can be robust to molecular noise, given the correct parameter choices. In this study, we focused on the effect of environmental noise on circadian clock evolution. To compare the idealized scenarios with natural conditions, we also evolved networks against a year-long time series of environmental radiometry data from Harvard Forest [15], where dawn and dusk change gradually and the light intensity fluctuates with the weather.

The networks were modeled as delay differential equations with parameters for light activation and for the signs, strengths, and timescales of gene-gene interactions. The choice of delays over mass action kinetics greatly reduces the number of parameters without being incompatible with biological systems [16–19]. For computational tractability, we limited the networks to no more than four genes. This limit was selected to allow a wide range of interlocking loop structures, comparable to the complexity of mechanistic circadian clock models. Over 10^8 network architectures were possible with four genes.

Network Analysis

The goal of using a genetic algorithm to optimize the topology and parameters was to create an ensemble of realistic networks. By strongly selecting for correct dawn and dusk gene expression, we removed most of the generated networks from further analysis. The absolute fitness and fitness distribution of the solutions varied significantly among scenarios, reflecting the challenges of the different environments and making it inappropriate to apply a single fitness threshold across scenarios. The 50 best performing solutions, out of 5,000 evolved, were therefore analyzed for each scenario. In general, biological networks might contain interactions that slightly increase fitness without being integral to function, so for the analysis of network structure, we exposed the functional network cores by iteratively removing the least important regulatory interaction or light input, stopping when the fitness would drop below 95% of its original value. The cores of the best performing networks are shown in Figures 1B–1F. For the single-photoperiod scenarios, the networks shown are representative of the 50 best solutions. What has evolved is a simple light-driven on/off switch for the dawn gene—an incoherent feed-forward loop with light as its input—with an additional delay for the dusk gene.

Figure 2 gives a summary of the evolved network structures and any sustained circadian oscillations. The simplest LD

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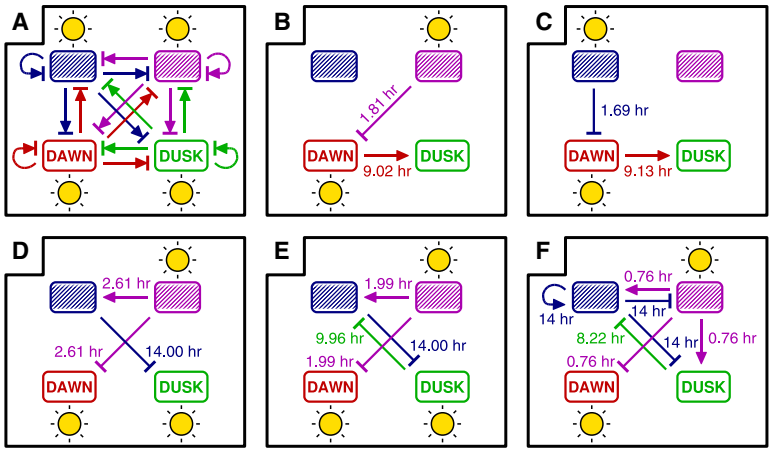

Figure 1. The Network Model

(A) The general form of the four-gene networks that we considered as candidates for generating circadian rhythms. Gene regulatory interactions may be positive, negative, or absent, and genes may be activated by light.

(B–F) The highest scoring network for five scenarios: (B) one photoperiod, (C) one photoperiod with noise, (D) multiple photoperiods, (E) multiple photoperiods with noise, and (F) one year of radiometry data. Gene interactions are shown with signs and delay times, and yellow suns denote light-activated expression. The designated dawn- and dusk-tracking genes are marked; the other two genes are interchangeable, so (B) and (C) are equivalent architectures. For both single-photoperiod scenarios (B and C), the networks shown represent the architecture of about 80% of the 50 best solutions (data not shown). The other 20% were functionally very similar, only replacing the positive regulation with a double negative. For multiple photoperiods (D), about 30% of the solutions looked like the one shown, whereas the last two scenarios did not use any one architecture for more than three of the solutions. The functional network cores were exposed by pruning of unimportant interactions (see text).

12:12 conditions only selected for delayed light responses, never oscillators, regardless of whether noise was added to the input. Extending the basic fitness function to multiple photoperiods had relatively little effect. The networks evolved few or no feedback loops, and circadian oscillations remained unlikely. In this scenario alone, we saw evidence of a tradeoff between light inputs and feedback loops, showing that under some circumstances, additional inputs are an alternative to increased structural complexity. However, combining multiple photoperiods with environmental noise eliminated that alternative strategy. Instead, the addition of noise led to a sharp increase in the number of feedback loops and in the probability of obtaining a circadian clock. Strikingly, networks faced with real environmental variations (Figure 3) evolved even more loops and light inputs and were most likely to exhibit circadian oscillations. Only in this scenario was the light level noisy during the day. Thus noise in the duration and level of the entraining light input signal appeared to favor greater complexity in the networks that timed gene expression.

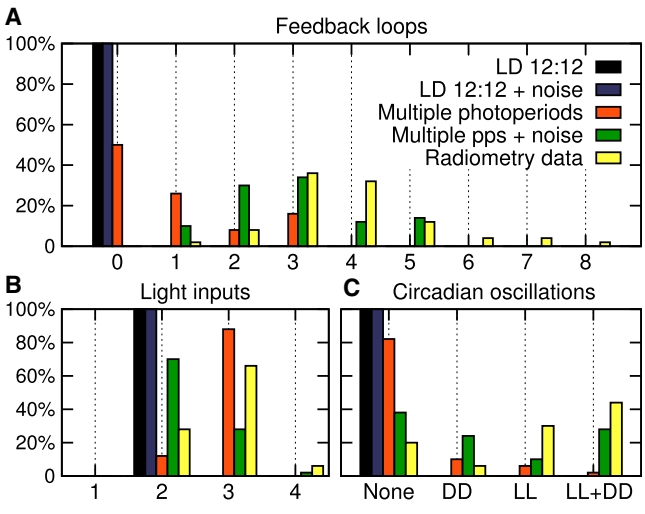

Figure 2. Complexity in Clock Networks Evolved under Different Environmental Input

The distribution of the number of feedback loops (A) and light inputs (B) in the functional cores of the top 50 networks from each scenario and the fraction of the networks that exhibit circadian oscillations only in constant light, darkness, or both (C). Increasingly realistic conditions led to more feedback loops, light inputs, and oscillations. The large numbers of light inputs selected under multiple photoperiods are discussed in the text.

Conclusions

A hallmark of circadian regulation is the ability to robustly adjust to different photoperiods despite unpredictable variations in temperature, light intensity, and other environmental parameters. By evolving systems in silico, we have explored the interactions between functional requirements on the timing of gene expression and robustness to noise in order to identify factors that can explain the ubiquity of multiloop circadian clocks. We have shown that seasonally changing photoperiods alone are insufficient to select for network complexity in a circadian system that can anticipate environmental transitions. However, when coupled with environmental noise, varying photoperiod strongly selects for complexity and gives rise to circadian clocks with multiple feedback loops and multiple light inputs, just as observed in nature.

Experimental Procedures

Network Model

The networks that we evolved are illustrated in Figure 1A. Transcription can be light activated, and genes might activate or repress the transcription of themselves and others. Posttranscriptional processes (including translation) give a discrete time delay of between 15 min and 14 hr. Following the time-averaged statistical treatment of Shea and Ackers [20], we modeled the system by four delay differential equations, each taking the form

$$\frac{dG_i}{dt} = S_i \frac{B_i + \Theta L_i o_{iL} + \sum_{j=1}^{4} a_{ij} o_{ij} \left(\frac{G_j(t-T_j)}{k_{ij}}\right)^2}{1 + B_i + \Theta L_i o_{iL} + \sum_{j=1}^{4} o_{ij} \left(\frac{G_j(t-T_j)}{k_{ij}}\right)^2} - D_i G_i(t),$$

where $G_i(t)$ is the level of gene i at time t, S_i its maximum transcription rate, B_i its basal expression level, and D_i its decay rate. Gene interactions are defined by the parameters o_{ij}, $a_{ij} \in \{0,1\}$. When $o_{ij} = 1$, there is repression ($a_{ij} = 0$) or activation ($a_{ij} = 1$) of gene i by gene j, with strength k_{ij} and time delay T_j. Similarly, if $o_{iL} = 1$, then light activates gene i with strength L_i when the entrainment signal $\Theta > 0$. The Hill coefficients for gene-gene interactions are fixed at 2. This model of a genetic network is highly simplified but nonetheless captures a wide range of network dynamics.

Fitness Function

Given parameter values and the input signal $\Theta(t)$, the G_i are determined as functions of time. The fitness score is based on the expression of one

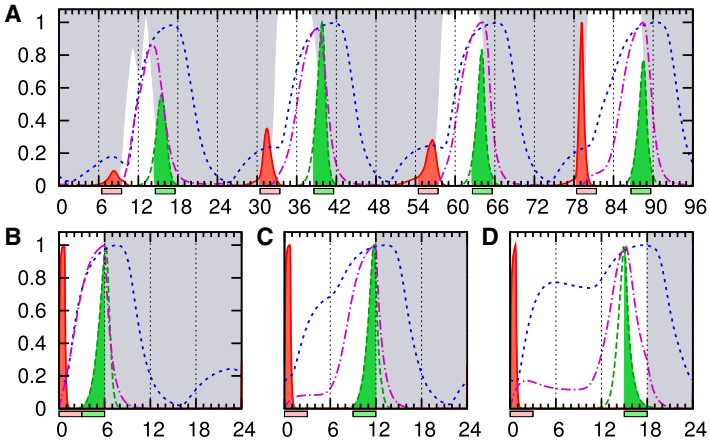

Figure 3. Network Dynamics with Real Environmental Input

Examples of network dynamics for the network of Figure 1F.

(A) A small part of the Harvard Forest radiometry data that the network was evolved against, and the corresponding gene expression time course, normalized to unit maximum. Periods of darkness are represented by gray shading. The target expression windows are indicated by red and green boxes for the dawn and dusk gene, respectively. The gene traces are plotted in the same colors as in Figure 1, with the parts matching the target expression windows shaded in red and green. Time $t = 0$ is midnight, not dawn, because there is no well-defined zeitgeber period and phase.

(B–D) The day length varies with the season between about 9 and 15 hr, but the network can be entrained to light/dark (LD) cycles with a wider range of photoperiods: LD 6:18 (B), LD 12:12 (C), and LD 18:6 (D). In (B)–(D), $t = 0$ is dawn.

gene in a 3 hr time window after dawn and of another gene in a similar window before dusk. The fitness for a single simulated day is then

$$f^{-1} \propto \left(\frac{\int_l^{l+3} G_1 dt}{\int_0^{24} G_1 dt}\right)^{-1} + \left(\frac{\int_{d-3}^{d} G_2 dt}{\int_0^{24} G_2 dt}\right)^{-1} + 0.01 \sum_{i=1}^{2} \left(\min\left(\frac{\int_0^{24} G_i dt}{1000}, 1\right)\right)^{-1} + 0.001 \left(\sum_{i,j} o_{ij} + \sum_i o_{iL}\right)^{-1},$$

normalized such that $0 \leq f \leq 1$. The first two terms describe the expression of the dawn and dusk genes in the time windows, relative to their totals, whereas the third term discourages very low expression levels. The last term is a small penalty on superfluous connections and light inputs, which mostly affects the simplest scenarios where the fitness differences between solutions are small. Without this term, feedback loops appeared in many networks even for the single-photoperiod scenarios, where they were not required for near perfect scores.

Simulations

To evaluate the fitness function for a given parameter set and light input signal, we implemented a delay differential equation solver in C++ using a fourth-order ordinary differential equation solver from the GNU Scientific Library (GSL) [21]. Hermite interpolation of the values and derivatives of the variables at the time points visited by the variable step-length ordinary differential equation (ODE) solver were used to provide system history for the delay terms and to evaluate the integrals of G_i. Each parameter set was thus always accompanied by its recent history, including current variable values. Simulations proceeded one day at a time, failing (reporting negative fitness) if more than 10^4 time steps were needed. Following any change, the system was converged toward a limit cycle for up to 20 days of identical light input, terminating early if end-of-day state or fitness score converged to within a 10^{-4} relative difference between several consecutive days. As a fallback, the worst fitness score of the last 15 days was reported.

Multiple Photoperiods and Noise

For multiphotoperiod scenarios, we used nine photoperiods between LD 6:18 and LD 18:6. The state was converged (as described above) following every photoperiod change. In scenarios with noise, the system ran for 24 days with dusk at nominal dusk ± 2 hr (flat distribution). The total fitness was the harmonic mean over the individual days. In the environmental data scenario, the system was converged against the first day of data, then simulated for a further 365 days. The input signal came from Harvard Forest data set HF102 (available at http://harvardforest.fas.harvard.edu/), specifically the hourly measurements of total incoming radiation for the year 2000. An arbitrary transformation was needed to give a level near 0 at night and saturated at 1 on sunny days. We used $\Theta = 0.5\tanh(x/30 - 2.5) + 0.5$ and interpolated between data points by a nonovershooting cubic spline (Figure 3). Nominal dawn and dusk at Harvard Forest, needed for the fitness scores, were computed using the date_sun_info function of the PHP programming language.

Genetic Algorithm

To evolve the networks, we used a real-coded genetic algorithm [22]. Our particular algorithm is described in detail in the Supplemental Data available online, and we give a brief summary here. In each generation, the bottom tenth of the individuals in a population of 50 parameter sets were replaced through cloning (including mutation of one or more parameters through multiplication by a random factor) or recombination (with new parameter values drawn from the vicinity of the two parents' values). The runs lasted between 1,500 and 25,000 generations, stopping when fitness could not be improved. Similar results were obtained from a different genetic algorithm in a separate implementation.

Circadian Oscillations

To test a network for circadian oscillations, we simulated the system for 10 days following entrainment in LD 12:12, switching to constant conditions in the first day. The expression levels of the dawn and dusk genes for days 4–8 were analyzed with fast Fourier transform nonlinear least squares (FFT-NLLS) [23] at confidence level 0.95. If any component with period between 15 and 35 hr was found, we considered the network to be a circadian oscillator. Figure S1 of the Supplemental Data shows the period and amplitude of the oscillations and that these are affected by the functional criteria used to evolve the networks. To remove very weakly oscillating networks, we required the root mean square (RMS) distance between the time course and a detrended version of the same time course to be at least 10% of the mean level for at least one gene.

Supplemental Data

Supplemental Data include Supplemental Experimental Procedures and one figure and can be found online at http://www.cell.com/current-biology/supplemental/S0960-9822(09)01704-7.

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Supplemental Data

Weather and Seasons Together

Demand Complex Biological Clocks

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Supplemental Experimental Procedures

Details of the Genetic Algorithm

The population consists of 50 parameter sets. In each generation, the 5 least fit are killed. Each new individual is created by cloning or recombination (50% chance). The parent(s) is/are drawn with probability proportional to 1/sqrt(fitness rank) (where the most fit has rank 1). In the case of cloning, the new individual is mutated *m* times, where *m* is a number between 1 and 6 drawn from an exponential distribution such that 1 is 10 times more likely than 6. Each mutation affects a randomly chosen parameter, with a real-valued parameter 10 times more likely to be picked than a Boolean one. A real-valued parameter is mutated by drawing a value *x* from a normal distribution (σ=1) until the parameter may be multiplied by 1.05^x without leaving the allowed range (see table, below). Boolean parameters are mutated by toggling. In the case of recombination, each Boolean parameter is taken from a random parent (with equal probability for both). Each real-value parameter is drawn from a normal distribution centred on the arithmetic mean of the parents' values (v_1+v_2), with standard deviation σ =sqrt(π/2)*abs(v_1-v_2), then clipped to within the allowed range.

The initial values for real-valued parameters are drawn from a flat distribution in log space. The Boolean parameters $o_{i\mathrm{L}}$ and a_{ij} are 0 or 1 with equal probability. For the first 500 generations, all the gene-gene interactions o_{ij} are clamped at 1 to promote the formation of useful connections.

The penalty on connections is not activated until generation 1000. After 1500 generations, the run terminates whenever the best fitness has not improved in 100 generations, and after 25000 generations it terminates regardless. For scenarios with non-deterministic fitness score (the ones with noise), in every generation a randomly drawn individual has its fitness recalculated.

Parameter	min	max
S_i	1e2	1e5
B_i	1e-3	1e3
L_i	1e-3	1e2
D_i	1e-1	1e1
T_i	0.25	14

Parameter	min	max
k_{ij}	1e-5	1e-1
$o_{i\mathrm{L}}$	0	1
o_{ij}	0	1
a_{ij}	0	1

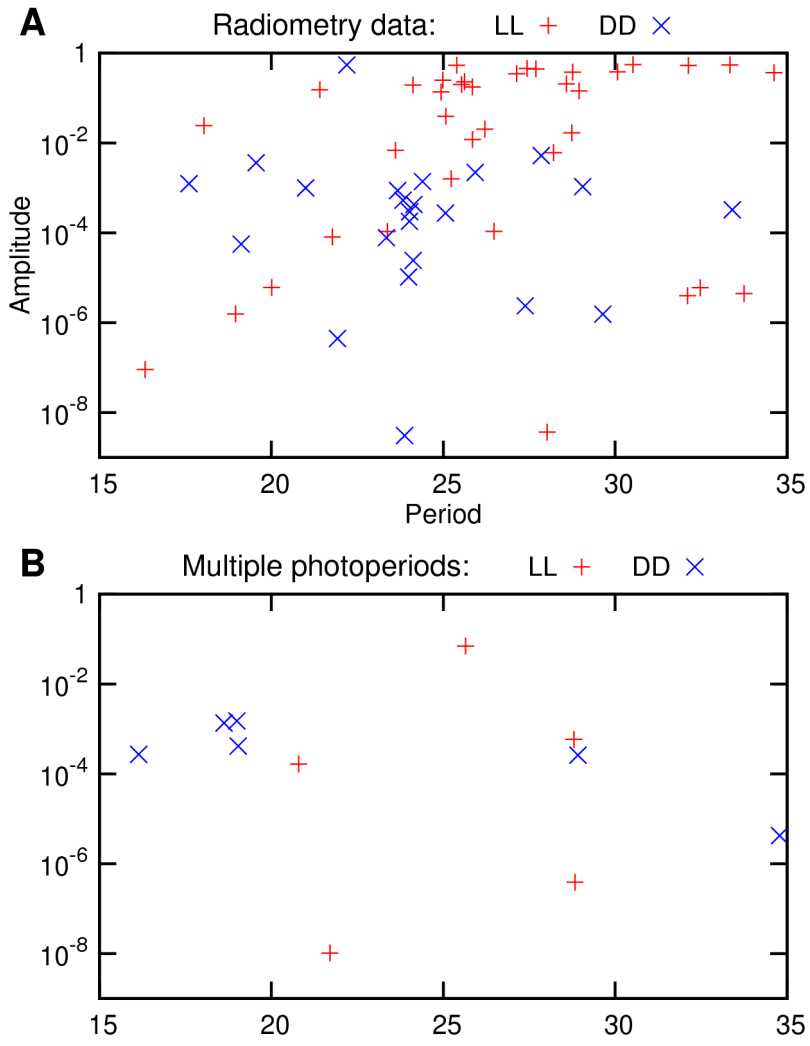

Figure S1. Properties of the Evolved Circadian Oscillators

The period and amplitude of the first component extracted by FFT-NLLS from the dusk gene expression in constant conditions, for the best-scoring networks from two scenarios: (A) Real radiometry data and (B) multiple photoperiods without noise. The more realistic conditions are seen to produce more networks with a period near 24 hours, especially in constant dark. There is also a clear trend towards longer-period oscillations in constant light. The time courses began with a day of LD 12:12 and were normalised to a peak value of 1, but the analysis used data from 2 to 6 days after release into LL or DD. To be classified as a circadian oscillator in Figure 2, a network with low-amplitude oscillations in the dusk gene would need stronger oscillations in some other gene(s).