

# Photoperiodic induction of synchronous flowering near the Equator

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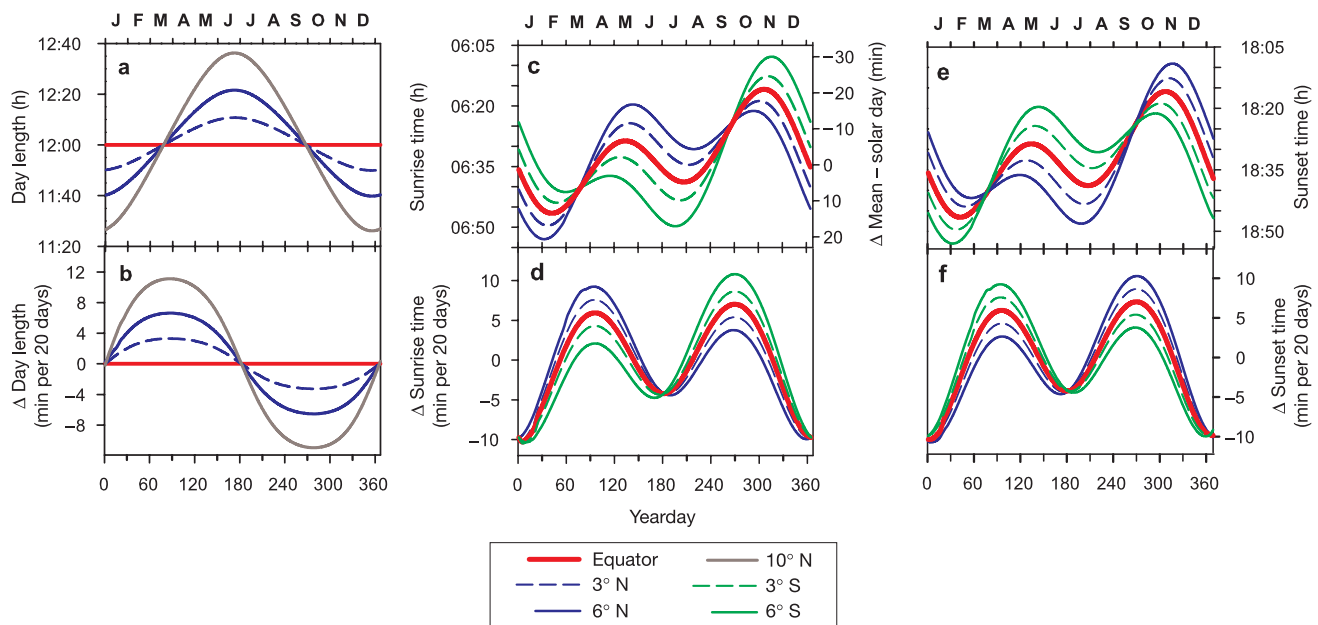
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In tropical rainforests, 30–65% of tree species grow at densities of less than one individual per hectare<sup>1</sup>. At these low population densities, successful cross-pollination relies on synchronous flowering. In rainforests with low climatic seasonality, photoperiodic control is the only reliable mechanism for inducing synchronous flowering<sup>2,3</sup>. This poses a problem because there is no variation in day length at the Equator. Here we propose a new mechanism of photoperiodic timekeeping based on the perception of variation in sunrise or sunset time, which explains and predicts the annually repeated, staggered, synchronous and bimodal flowering of many tree species in Amazonian rainforests near the Equator.

Seasonal variation in day length is the only environmental signal that is constant from year to year; it is independent of seasonal and inter-annual variation in climate and is capable of inducing synchronous developmental change in conspecific trees at the same time every year<sup>2–4</sup>. Photoperiodic control of tropical tree

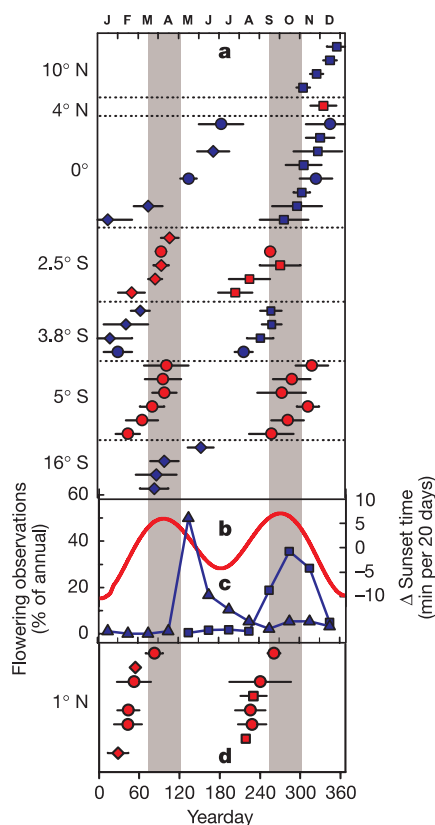
development has been overlooked, but recent studies have shown that photoperiodic induction of synchronous vegetative bud break or flowering is common in tropical forests around the globe<sup>3–6</sup>. At tropical latitudes, changes in day length large enough to affect plant development occur only around the equinoxes<sup>3–7</sup> (Fig. 1a, b). A single annual period of synchronous flowering 1–2 months after the autumn equinox indicates the induction of flowering in ‘short-day plants’ in response to declining day length<sup>4</sup> (Fig. 2a; 10° N, 16° S). Flowering of ‘long-day plants’ and vegetative bud break induced by increasing day length occur after the spring equinox<sup>3,5–7</sup>. Such flowering periods are six months out of phase between the Northern and Southern Hemispheres<sup>4,7</sup> (Fig. 2a; compare 10° N and 16° S). In tropical semi-deciduous forests at 10° N and 16° S, photoperiodic control causes the synchronous onset of flowering in all trees of certain species during the same two-week period each year<sup>4</sup>, as indicated by small standard deviations in mean flowering time (Fig. 2a; 10° N).

At 4° N, near Cali, Colombia, flowering of more than 100 *Montanoa quadrangularis* trees was observed during the same two-week period over three consecutive years<sup>8</sup> (Fig. 2a; 4° N). This raised the question of how such synchronized flowering near the Equator could be induced by day length changes of <30 min year<sup>−1</sup> (Fig. 1a) or <4 min over 20 days (Fig. 1b). At the Equator, where day length is constant throughout the year (Fig. 1a, red line), the times of sunrise and sunset (Fig. 1c, e, red lines) vary by 30 min over the course of the year (<http://www.geocities.com/jllammi/>) and therefore might act as a celestial flowering signal. Annual variation of sunrise time at the Equator corresponds to the difference between apparent solar time measured by a sundial and mean time measured by chronometers<sup>9</sup> (‘equation of time’; Fig. 1c, red line, right y-axis). In contrast to seasonal variation in day length (Fig. 1a), sunrise time has two annual maxima and minima, corresponding to the largest delays or advances of solar time relative to mean time (Fig. 1c, red line). The asymmetric, bimodal shape of this curve is generated by the combination of two properties of the Earth’s orbit around the Sun: its elliptic (rather than circular) shape and the tilt of the Earth’s axis relative to its orbit<sup>9</sup> (see Supplementary Discussion 1). With increasing latitude, the asymmetry of the



**Figure 1** Seasonal variation in day length, sunrise and sunset time near the Equator. Day length (a), sunrise (c) and sunset (e) times obtained from the internet (<http://www.geocities.com/jllammi/>) were used to calculate running 20-day differences (b, d, f).

The equation of time equals seasonal variation in sunrise time at the Equator (c, right y-axis, red line).



**Figure 2** Synchronous, bimodal flowering and bud break in tropical forests at low latitudes. **a**, Mean times of synchronous flowering in 41 representative tree species observed in seven tropical forests ranging from 10°N to 16°S. For each forest site, the duration of the observation period and the author name for any unpublished field observations are given. Species names (for each site, from left to right and top to bottom; diamonds indicate spring-flowering species, squares indicate autumn-flowering species, circles indicate species flowering in the spring or autumn) are followed by the number of trees used to calculate mean flowering time and standard deviation (error bars). 10°N: Tropical semi-deciduous forest in Guanacaste, Costa Rica<sup>4</sup> (3 yr); *Cochlospermum vitifolium* (>100), *Cordia alliodora* (>100), *Calycophyllum candidissimum* (>100), *Bixa orellana* (10). 4°N: Tropical montane forest in El Dovio, Valle de Cauca, Colombia<sup>8</sup> (2 yr); *Montanoa quadrangularis* (113). 0°: Rainforest at Parque Nacional Chiribiquete, Caquetá, Colombia (D.N., 3.5 yr); *Clusia chiribiquetensis* (143), *Peltogyne catingae* (12), *Pouteria* sp. (26), *Manilkara bidentata* (19), *Iryanthera obovata* (15), *Mollia speciosa* (13), *Clathrotropis macrocarpa* (287), *Erisma laurifolium* (64), *Micropholis* sp. (13), *Protium guianense* (11), *Iryanthera tricornis* (13). 2.5°S: Rainforest at Manaus, Amazonia, Brazil (S.S.R., 3 yr); *Miconia longispicata* (10), *M. pyriformis* (8), *M. elaeagnoides* (12), *M. regelii* (9), *M. burchellii* (31), *M. egensis* (8), *M. myriantha* (7), *M. dispar* (9). 3.8°S: Rainforest at Parque Nacional Amacayacu, Amazonas, Colombia (A.T., 2.2 yr); *Miconia* cf. *cayumbensis* (11), *Randia ruiziana* (4), *Swartzia auriculata* (7), *Coussarea paniculata* (7), *Henriettea stellaris* (9), *Psychotria* cf. *capitata* (6), *Miconia sprucei* (3). 5°S: Rainforest at Arboletum Jenaro Herrera, Requena, Loreto, Peru<sup>15,16</sup> (8 yr); *Caryocar glabrum* (37), *Quararibea ochrocalyx* (17), *Helicostylis scabra* (9), *Swartzia cuspidata* (6), *Eschweilera tessmannii* (22), *Diclinanona tessmannii* (24). 16°S: Tropical semi-deciduous forest at Lomerio, Ñuflo de Chavez, Santa Cruz, Bolivia<sup>4,17</sup> (3 yr); *Vochysia mapiensis* (10), *Calycophyllum multiflorum* (99), *Pterogyne nitens* (87), *Centrolobium microchaete* (84). **b**, Seasonal variation in sunset time. Vertical grey bars indicate periods of maximum advance in sunset time. **c**, Flowering of eight spring-flowering (triangles) and 27 autumn-flowering species (squares) in the equatorial rainforest at Parque Nacional Chiribiquete, Caquetá, Colombia (D.N.). **d**, Vegetative bud break observed over ten years in individual trees of deciduous or leaf-exchanging species in the Singapore Botanical Garden at 1°N<sup>10</sup>. Four species exchanged leaves twice a year (circles). Top to bottom: *Couroupita guianensis*, *Parkia javanica*, *Ficus variegata*, *Lecythis* sp., *Peltophorum pterocarpum*, *Terminalia catappa*, *Cedrela glaziovii*, *Kigelia pinnata*.

curve increases as the effects of the equation of time combine with progressively larger seasonal variation in day length (Fig. 1c).

If induced by bimodal variation in sunrise or sunset time, synchronous flowering near the Equator should occur after both equinoxes, when these variables change fastest (Fig. 1d). Flowering synchrony observed in four 2–8-yr phenological surveys of thousands of trees in Amazonian rainforests within 5° of the Equator is generally less precise than at higher latitudes; this is probably because of the marginal photoperiodic signal. Species with synchronous flowering were therefore identified as those for which flowering occurred at the same time in different years and >75% of flowering observations were made within three-month periods following several months with low or no flowering activity (Fig. 2c; see Supplementary Information). Most of these species flowered during the predicted periods (Fig. 2a–c, grey bars). Two annual fruiting periods have also been observed in other species (A.T., unpublished observation). The striking coincidence between the two annual maxima of advancing sunrise/sunset time (at the equinoxes) and the onset of two annual flowering periods in equatorial rainforests provides strong evidence for the importance of advancing sunrise/sunset time in photoperiodic timekeeping. There is no other bimodal environmental signal in these forests that occurs at precisely the same time each year. No climatic cue, such as rainfall or temperature, can explain an identical sequence of species' flowering times year after year. In South America, bimodal flowering caused by bimodal rainfall occurs only in seasonally dry forests at ~4°N, and its timing varies from year to year with seasonal rainfall (see Supplementary Discussion 2).

The highly synchronous, bimodal vegetative bud break observed over ten years in deciduous and leaf-exchanging tree species in the equatorial climate of Singapore at 1°N is also indicative of photoperiodic control<sup>10</sup> (Fig. 2d). In some of these species, increasing day length is known to induce synchronous vegetative bud break after the spring equinox in mid-latitude tropical forests ('spring flushing'<sup>3</sup>; Fig. 2d, *Parkia*, *Cedrela*). Synchronous bud break soon after the summer and winter solstices suggests that seasonal delays in sunrise or sunset time, rather than changing day length, induce the breaking of bud dormancy (Figs 1c and 2d). In contrast, vegetative development of *Bombax malabaricum* is asynchronous in Singapore, but synchronous at 5°N and 5°S<sup>10</sup>, indicating that perception of changes in sunset time may be less sensitive in this species. Similarly, at higher tropical latitudes, bud break of *Bursera* is induced by increasing day length, but on the Galapagos Islands (0°) it is triggered by the first rains after seasonal drought (A.T., unpublished observation).

Plants use different pigment systems to perceive the large changes in light intensity at dawn and dusk<sup>2,11</sup>. They measure changes in length of the solar day by relating the signals at dawn and dusk to the phases of their endogenous circadian clock. This process is not well understood<sup>2,11,12</sup>, but it seems likely that changes in sunrise or sunset time could also be measured by this mechanism. The minimum change in day length required to induce flowering is not known. Experiments with the short-day plant rice (*Oryza sativa*) at 2°N suggest that a 15-min change in day length accumulated over a few weeks may be sufficient<sup>13</sup>. To assess the potentially inductive changes in day length versus sunrise and sunset time near the Equator, we calculated the cumulative changes of these variables over an assumed 20-day induction period (Fig. 1b, d, f; see Supplementary Table). Around the autumn equinox, the advance in sunrise time decreases with increasing latitude, but the advance in sunset time increases (compare Fig. 1d and f: 6°N and 3°N at yearday 270, 6°N and 3°S at yearday 90). These observations suggest that at latitudes between 6°N and S, cumulative changes in sunset time rather than the much smaller changes in sunrise time or day length induce autumn-flowering of short-day plants (Fig. 2a; 4°N to 5°S). Correspondingly, at and near the Equator, flowering around the autumn equinox appears to be more synchronized than around the

spring equinox (Fig. 2a; 0° and 2° S), when the advances in sunset time are slightly smaller (7 and 7.6 min versus 5.9 and 5.4 min). Implicitly, cumulative changes in sunset time of 5–7 min over 20 days are sufficient to induce flowering at the Equator.

In view of the small maximum changes in sunset time near the Equator (5–7 min over 20 days) it seems likely that signals sufficiently large to trigger flowering or vegetative bud break occur only during periods of maximum change (Fig. 1 d, f), that is, around the equinoxes (Fig. 2, grey bars) or solstices (Fig. 2d). If so, the observed staggered flowering times (Fig. 2a) could be primarily attributable to differences in the duration of flower development induced by perception of the photoperiodic signal. Flowering during an inductive period might indicate rapid flower emergence from resting buds, as in several *Miconia* species (Fig. 2a; 2.5–3.8° S). In other species, trees may flower months after the inductive period because the flowering signal causes the vegetative shoot apex to change into a large, branched inflorescence supporting many flowers<sup>4</sup> (Fig. 2a; 4° N *Montanoa*). It remains to be explained why some species flower only during or shortly before one of the two induction periods and why many trees do not flower every year.

This is the first study that both confirms synchronous flowering in rainforest tree species near the Equator and proposes a timing mechanism. Synchronous flowering at the same time each year has long been noticed in Amazonian trees such as *Miconia* (Fig. 2a; 2.5° S), but studies of the phenomenon focused on the evolutionary consequences of staggered synchronous fruiting, for example, for frugivorous birds<sup>14</sup>. Photoperiodic control of vegetative development and flowering in tropical trees evolved in response to different adaptive pressures<sup>3,5,6</sup>. In tropical rainforests with an equitable climate, it may have evolved in response to the need to synchronize flowering to achieve cross-pollination in spite of low population densities. □

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**Supplementary Information** accompanies the paper on [www.nature.com/nature](http://www.nature.com/nature).

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## Gene transfer to plants by diverse species of bacteria

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*Agrobacterium* is widely considered to be the only bacterial genus capable of transferring genes to plants. When suitably modified, *Agrobacterium* has become the most effective vector for gene transfer in plant biotechnology<sup>1</sup>. However, the complexity of the patent landscape<sup>2</sup> has created both real and perceived obstacles to the effective use of this technology for agricultural improvements by many public and private organizations worldwide. Here we show that several species of bacteria outside the *Agrobacterium* genus can be modified to mediate gene transfer to a number of diverse plants. These plant-associated symbiotic bacteria were made competent for gene transfer by acquisition of both a disarmed Ti plasmid and a suitable binary vector. This alternative to *Agrobacterium*-mediated technology for crop improvement, in addition to affording a versatile 'open source' platform for plant biotechnology, may lead to new uses of natural bacteria–plant interactions to achieve plant transformation.

*Agrobacterium tumefaciens* is a ubiquitous soil bacterium that induces galls on plants. The discovery that this gall formation is due to integration into the plant genome of bacterial DNA (T-DNA) laid the foundations for plant biotechnology<sup>3</sup>. The T-DNA is part of the ~200 kb Ti (tumour-inducing) plasmid, which also encodes functions for Ti plasmid conjugation, opine metabolism and the initiation, transfer and processing of the T-DNA<sup>4,5</sup>. Before the discovery of the Ti plasmid, gall-inducing ability was shown to be transferable to non-virulent *Agrobacteria* and to *Rhizobium leguminosarum*<sup>6</sup>. Ti plasmid transfer to *Rhizobium trifolii* and *Phyllobacterium myrsinacearum* resulted in strains that caused galls on some plants<sup>7,8</sup>, but a *Sinorhizobium meliloti* strain containing a Ti plasmid was not tumorigenic<sup>9</sup>. Although these experiments showed that close relatives of *Agrobacterium* could harbour the Ti plasmid, no direct molecular evidence of gene transfer to plants by these bacteria was reported, leaving open the possibility that gall formation may have resulted from hormonal perturbations in the host plant unrelated to DNA transfer<sup>10</sup>. Indeed, a disarmed Ti plasmid and binary vector were introduced into a bacterial isolate apparently related to *Phyllobacterium* spp. for the purpose of tobacco inoculation<sup>11</sup>, and although galls resulted from production of auxin by *Phyllobacterium*, these galls were morphologically different from those produced by an *Agrobacterium*-transformed plant through gene transfer; moreover, evidence of gene transfer was sought but not found. Accordingly, the scientific community has focused on *Agrobacterium* as a vehicle for gene transfer; the vast majority of patent claims regarding biological plant transformation explicitly refer to *Agrobacterium*<sup>2</sup>. A recent proposal suggesting that *A. tumefaciens* be reclassified as *Rhizobium radiobacter* has been widely disputed<sup>12</sup>, although *Agrobacterium* is clearly closely related to *Rhizobium*. However there is little doubt that *Agrobacterium*, *Sinorhizobium* and *Mesorhizobium* are in distinct phylogenetic clades and their genomic organization differs considerably<sup>4,13</sup>.