SUPPLEMENTARY DATA S1

Etide, Marine tide, and Earthtide

Etide

The Etide program was designed for use in geodesy. Based on 50 astronomical and geophysical parameters (Longman 1959), some of which are either constants (e.g., mass of the Earth, mass of the Sun, ...) or easily computable (e.g., distance of the Earth from the Sun, ...), and others whose variables can be derived by making use of a set of 34 interlinked equations, the program provides estimates, at 15 min intervals, of the lunisolar tidal acceleration at any given location upon the Earth's surface for any calendar date, past or future. The estimated values are in units of μGals, where 1 Gal = 1 cm s^{-2}, and the Earthly gravitational force 1 g = 9.81 x 10^8 μGals. They represent the variations ($\pm \delta$g) in the Earth's gravitational attraction at a given location due to the lunisolar tidal force which deforms the body of the Earth, raising or lowering its surface. For example, a rise or fall of the Earth's surface of ±1 cm leads to a change of ±3.1 μGals, respectively, The tidal acceleration itself, as experienced on Earth, is the result of the motions of Earth and Moon relative to the Sun. The ever-changing positions of these three bodies relative to one another result in a diurnal or semi-diurnal wave of elastic deformation that passes throughout the Earth's surface as an 'Earth-tide' (http://en.wikipedia.org/wiki/Earth_tide). The gravitational attraction exerted by the Moon accounts for 60% of this deformation, the remaining 40% is due to the Sun.

The Etide program estimates the vertical component of the lunisolar tidal variation. European locations on the Earth's surface may typically rise and fall by a total of approx. 60 cm during

the solar day, given a typical maximal daily amplitudinal variation of 200 μGal. A factor

which limits this variation is the Earth's degree of rigidity, denoted by an 'elasticity factor'.

In the present case, this is set at 1.16. The horizontal component is small, and is ignored in

the present computations of Etide values. It is not negligible, however, for it has been

estimated that, as a result of the Moon's motion across the sky, the Empire State Building in

New York, USA, and the Eiffel Tower in Paris, France, structures separated horizontally by

approx. 5840 Km, range 19 m in distance from each other each day

(http://www.northernontario.org/StuffOfLife/CraftHaven/Selenology.htm).

A consequence of the vertical rise and fall of the Earth's surface during the passage of the

Earthtide is that μGals are temporarily added to or subtracted from the gravitational

acceleration attraction of the Earth at the locality in question. The amount of variation of the

gravitational attraction ($1\,g \pm \delta g$) is small and is usually regarded as unlikely to have any

appreciable effect on a living organism. Nevertheless, potentially significant biological

results from such minute forces have been discussed from a theoretical point of view by

Persinger (2014). The putative consequences of the relationship between the Etide

timecourses and timecourses of biological processes are indicated by the changes in the

direction of leaf movements. It is hypothesised that the critical point in this relationship is the

rate of change of δg, i.e., $\delta(\delta g)$, especially when $\delta(\delta g) = 0$; and that this turning point values,

$\delta(\delta g) = 0$, is the effective impulse for an alteration of biological growth.

Marine tide

Although the Etide values devolve to geophysical properties of the Earth, they also reflect

and stand proxy for the gravitational attraction of Sun and Moon as it is experienced upon

Earth, the pull of the Moon being much greater than that of the Sun, due to the Moon's greater proximity to the Earth. The influence of the lunisolar tidal acceleration is exemplified by the rise and fall of marine tides. Examples of this interrelationship are illustrated in Appendix Figs A1(A), 1(B). The marine tidal data are for the Port of Brest, France, which is located at a longitude similar to many of the sites inland and east of Brest, at which observations of leaf movements described in the text were made.

The timecourses of the marine tide and the contemporaneous δg values, estimated from the Etide program resemble those seen in the figures in the main text, where timecourses of leaf movements are plotted together with the respective Etide timecourses. The example in Appendix Fig. A1 shows that, at the commencement of the lunar month, at New Moon (NM), the daily maxima (peaks – labelled A and B in the panels relating to δg) and minima (troughs) of the daily Etide values coincide, respectively, with high and low marine tides (red and green arrows in Appendix Fig. A1(A)). In this example, the relationship is 'in phase'. However, there are days in the early portion of the lunar month, around First Quarter (FQ) Moon phase, when the phase relationship between Etide and marine tide is disturbed, or even reversed; that is, the relationship changes to 'in antiphase', with Etide peaks and troughs now coinciding with low and high marine tides, respectively (blue and yellow arrows in Appendix Fig. A1(A)). Sometimes marine tides are high when there is no corresponding peak (or trough) of Etide. It is as though the marine system has an inertial oscillation of its own but which, in the long term, is nevertheless entrained by the lunar rhythm. By the time of Full Moon (FM), the 'in phase' relationship has been restored (red and green arrows in Appendix Fig. A1(B)), but reverts to 'in antiphase' once more, at around Last Quarter (LQ) Moon (blue and yellow arrows in Appendix Fig. A1(B)). Subsequently, there is a restoration of the 'in phase' relationship of Etide and marine tide as the next NM approaches (not shown). Also

noticeable is the changing amplitude of the marine tide across the lunar month, this being mirrored in the changing amplitude of the daily values of δg in the Etide timecourse.

Earthtide

A second example, which shows a more direct relationship to the output from the Etide program than does the timecourse and structure of the marine tide (this latter being subject to local adjustments due to water currents, coastline topography, etc.), is the elastic deformation of the Earth itself (Appendix Fig. A2). This is the Earthtide of which the Etide estimates are a realisation. Measurements of the Earthtidal deformation were made over a number of years by Albert Michelson and Henry Gale (1919), working at the Yerkes Observatory, Williams Bay, WI, USA. Their approach was to record the changes, with time, of the number of interference fringes observed by interferometry upon an approx. 0.5 mm-thick water film that covered a mirrored surface. The progress of the Earthtide brought about changes in the thickness of the water film, as registered by a shift in the number of fringes. Each increment of one fringe corresponded to an increment of water height of 0.639 μm.

The water reservoir for these optical observations was a closed system of horizontal underground pipes oriented in the North-South and East-West directions. Plots of fringe number (F) on selected days at four different phases of the Moon, during the collection of a month-long stream of data (from 23/03/1917 to 21/04/1917) at the North-South-oriented observation point, are plotted in Appendix Fig. A2 together with the contemporaneous values (δg) estimated by the Etide program. The visualisation of the two timecourses suggests a close interrelationship. This is not unexpected because the interferometry system recorded the vertical change in height of the superficial water film, standing proxy for the changes in

height of the Earth's surface, during the passage of the Earthtide, as experienced at the Yerkes observation site.

The relationship between the timecourses of fringe number and Etide value, is consistently shown to be 'in antiphase' (Appendix Fig. A2), though the nature of this phase relationship is perhaps a matter of methodology. However, the consistency of the relationship is not altered by the lunar phase. At NM, the relative positions of the three bodies [Earth-Moon-Sun] is in that order, whereas at FM the positional order is [Moon-Earth-Sun]. In this latter configuration the Moon is at the opposite side of the Earth with respect to the Sun, whereas in the former configuration, the Moon is between the Earth and the Sun. The elastic deformation of the Earth affects the whole of that body, not just the part of its surface which faces the Moon. In the configuration [Earth-Moon-Sun], the Moon exerts its gravitational attraction upon the surface of the Earth in the same direction as the Sun. By contrast, in the opposing arrangement [Moon-Earth-Sun], the Moon exerts a gravitational attraction in opposition to the Sun.

It is noticeable that the Earthly deformation monitored by Michelson and Gale's optical set-up does not precisely track the theoretical expectation, as judged from the output of the Etide program: the general oscillatory pattern of the Etide is followed, but not the minor subdivision of the Etide daily maximum into its component A and B peaks (Appendix Fig. A2). As will be seen later in the main text figures, the same feature is found with respect to the oscillatory leaf movements and their ability to track the Etide timecourse.
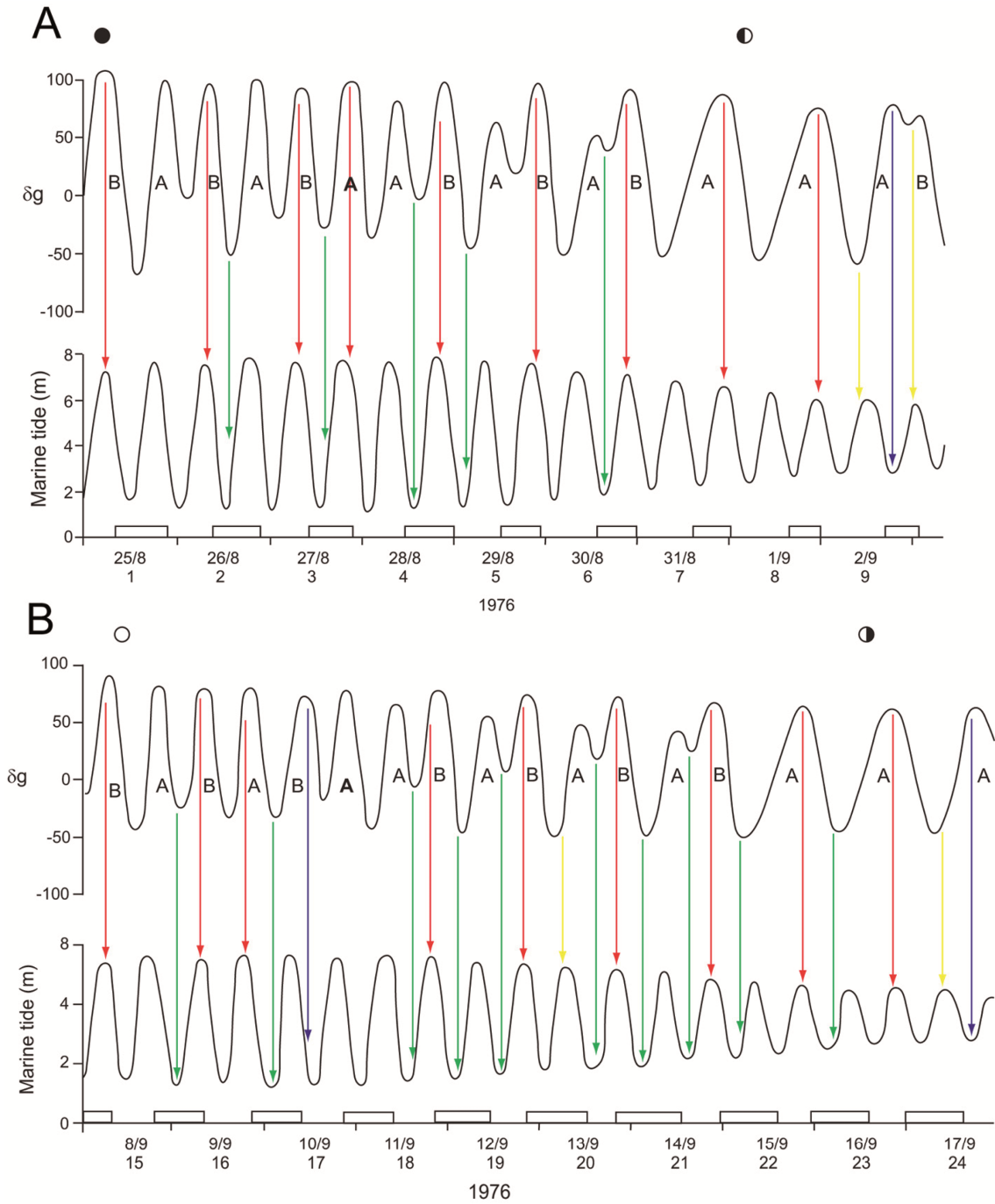
FIG. A1 A, B. Relationship between the lunisolar tidal acceleration (δg), estimated by the Etide program, and the rise and fall of the marine tide. Both timecourses apply to the location of the port of Brest, Breton Peninsula, France. Dates are chosen in August-September 1976: A) from the time of NM until FQ Moon, and B) from FM to LQ Moon. The time axis states the day and month beneath which is placed the day number of the lunar month. Lunar day 1

corresponds to the date of New Moon. Open bars on the time axis indicate the time between

Moonrise and Moonset. The lunisolar tidal structure is basically bimodal, with peaks [A,B],

but there are also unimodal days consisting of a single peak [**A**], as well as other [A] peaks,

which are composite [A,B] peaks. Arrows indicate the correspondences between maxima or

minima Etide and marine tide values. Respectively, these correspondences are – Red arrows:

High tide to High tide; Green arrows: Low tide to Low tide; Blue arrows: High tide to Low

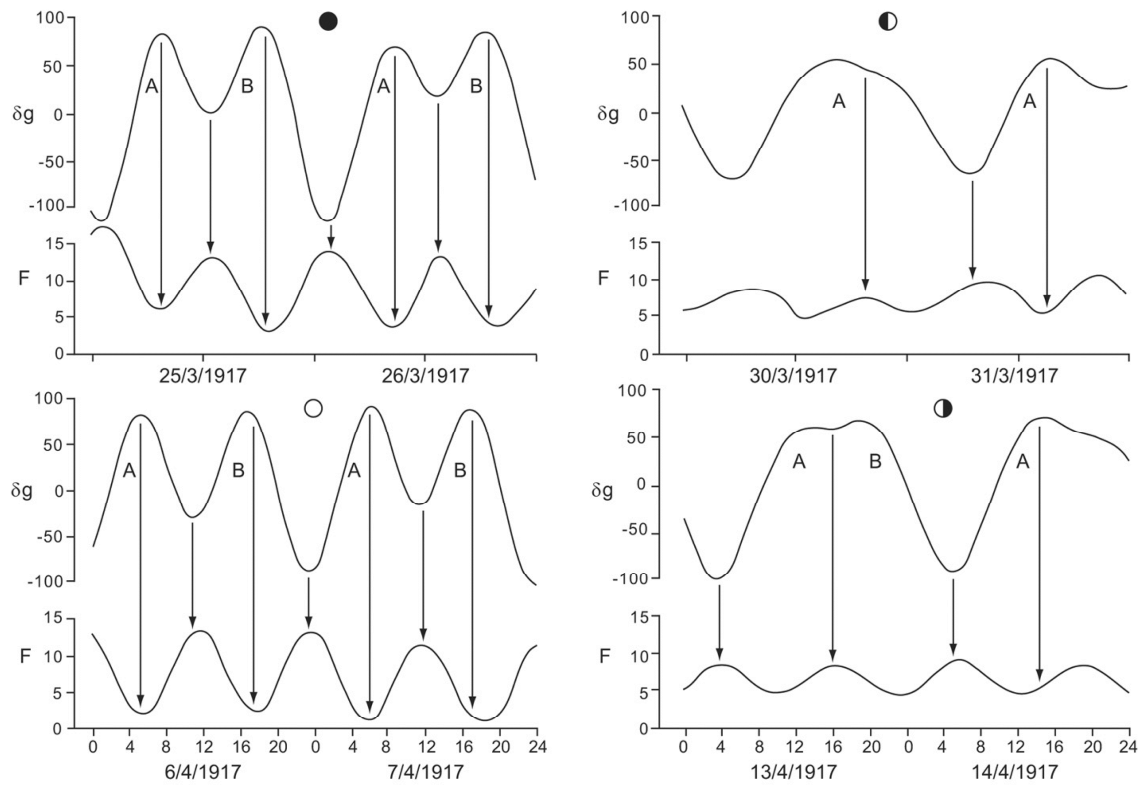tide; Yellow arrows: Low tide to High tide.

FIG. A2. Correspondences between the lunisolar tidal acceleration (δg) and the Earthtide

measured by interferometry and recorded as a timeseries of interference fringe numbers (F).

The latter values were obtained by Michelson and Gale (1919) at Yerkes Observatory, WI,

USA, and the present curves have been redrawn from Fig. 2 of their publication. Values for

two days at each of the four phases of the Moon, NM, FQ, FM and LQ, are shown. Lunisolar (δg) peaks [A,B] are marked, as in Appendix Fig. A1 A, B. Vertical arrows indicate correspondences between maxima and minima (turning points) of the Etide δg estimates and the peaks and troughs of the Earthtide F values.

SUPPLEMENTARY DATA S2

Sun, Moon and Etide

As described in Appendix 1, the timecourse of the Etide variation, δg, is analogous to the progress of the Earthtide at a particular location upon the Earth's surface, as a consequence of the orbits of the Earth and Moon. Also apparent is a relationship between the general structure of the Etide timecourse and the phase of the Moon. As we shall see, this helps explain the alternating relationships between leaftide and Etide at different times of the lunar month.

Each lunar month (or lunation) of approx. 29.5 d commences on the calendar date of New Moon (or Dark Moon). This date therefore corresponds to lunar day no. 1 of each complete lunar month of a solar year. The precise time during the solar day when the lunar month commences is variable, however. The mean length of a lunar 'day' has been estimated (for the year 1989) as 24.848 h by Mikulecký and Pittich (2012, unpub. res.).

In many of the figures in the main text, the times of the rising and setting of Sun and Moon are given because these details are needed in order to understand their relationships with the Etide timecourse. The relevant data that apply to the location at Delft, Netherlands, at the time when Kleinhoonte (1929) was making observations on bean leaf movements (in the year

1926) and are sufficient to show how this inter-relationship of the three mentioned bodies of Sun, Moon and Earth develops. Suitable lunar dates for appraisal are those around the solstices and equinoxes, when Moonrise and Sunrise often coincide. Thus, on 14/3/1926, at the beginning of lunar month no. 3 (Vernal Equinox occurs during this month), Moonrise occurs 39 min before Sunrise. Accordingly, the Moon is present in the sky during daytime as a 'dark' Moon, reaching an altitude of 32° by 1300 h before setting at 1912 h, at which time 1% of its surface has become illuminated. Because the time of Moonrise advances 0.5-1.5 h each day as the month progresses, two weeks later, at Full Moon, Moonrise now approximately coincides with Sunset. The Moon is then visible in the night sky. On the date of the next New Moon, on 4/4/1926, there is still a near-coincidence of Sunrise and Moonrise, but now Moonrise occurs 83 min before Sunrise. The timecourse of the Etide over the course of lunar month no. 3 shows that maxima (peaks) and minima (troughs) of $\pm\delta g$ values (ranging from +100 to −60 µGal) during each 24.0-h solar day are closely associated with the times of Moonrise and Moonset. At the two Quarter Moon phases (FQ and LQ) a major minimum of δg (−50 µGal) is associated with Sunset. From these and other data, it is evident that the relative positions of the Sun and Moon as these bodies rise above or sink below the horizon during the Solar day determine the oscillatory features of the Etide at any particular geographical location.

SUPPLEMENTARY DATA S3

FIG. S1. Gerrit Brouwer. A portrait photograph taken in 1959 (with permission of Natuurmonumenten, 's-Graveland, Netherlands).

FIG. S2. Anthonia Kleinhoonte. Photograph taken in her laboratory at Delft (with permission of the Delft School of Microbiology Archives, Dept of Biotechnology, Delft University of Technology).

FIG. S3. Anthonia Kleinhoonte (left) and Rose Stoppel (right). From a photograph taken in 1939 at a meeting of the International Society for Rhythm Research, Utrecht.

SUPPLEMENTARY DATA S4

Alternative timings of leaf movement at Akureyri, Iceland

It would have been interesting if observations of leaf movements at Akureyri had been made on the same date but in other years besides 1925, in 1926 or 1928, for example, for although the lengths of night and day were the same as on the date in question, June 25 1925, the intervals between Moonrise and Moonset were different. Thus, on this date in 1926, the interval was approx. 3.25 h; in 1928 it was approx. 11.25 h; and in 1925 it was 18.5 h. This

variation occurs because, in the different years, the date June 25 falls at different positions within the lunar month: in 1925, June 25 is lunar day 5 of lunar month 6; in 1926, it is lunar day 16 (FM); in 1928, it is lunar day 9. In Fig. 4 in the main text (for this year, 1925), the Etide minima are associated with descents of the leaf from the 'up' position. But in 1926 and 1928, the respective Etide minima occur 2 h and 6 h earlier than it does in 1925. Because of the altered spacing of Etide maxima and minima on this date in each of these three years, the relative timings of leaf ascents and descents might be changed, even though the period of leaf movement, τ, might remain constant.

SUPPLEMENTARY DATA S5

Etide during the lunar month

To understand the changing phase relationships between the rhythms of leaf movement and Etide, it is necessary to study the structure of the timecourse of δg prepared by the Etide program. As indicated in the main text and its accompanying figures, the leaftide/Etide phase relationship changes around the time of the Quarter Moon phases (Figs 2, 3, 6 and 7). At the latitude of Delft, where relevant observations were made, the Etide on one day near the date of each of these Quarter Moon phases is marked by a singular Etide peak (**A** in the δg panels of Figs 2 and 3). In Fig. 2 of the maintext, the **A** peak occurs on lunar day 25, two days after LQ Moon, and in Fig. 3, the **A** peak straddles lunar days 8-9, occurring one day before FQ Moon. Even though the presence of the singular **A** peak does not affect the period of the Etides on these dates, there is nevertheless a progressive development of the Etide structure on the days which follow. This restructuring of the Etide has consequences for the leaftide/Etide relationship. As seen in Fig. 2, the B peak of the [A,B] pair of Etide peaks, the paired peaks being characteristic of the days before and after NM and FM, diminishes and finally disappears two days after LQ Moon to result in the mentioned **A** peak on lunar day 25. On the days that follow, a new series of paired [A,B] peaks is established. In the case of the lunar days shown in Fig. 3, much the same restructuring occurs so that, following the loss of the B peak from the [A,B] pair, a singular **A** peak is present one day before FQ Moon. Then, a new series of [A,B] peaks emerges over the next 18 days. During this time, three further A peaks are present which, because of their broad span (unlike the acute, singular **A** peak), appear to be composite [A,B] peaks.

The complete structures of the Etides for these two examples, estimated at Delft during the lunar months in question, are as follows:

Lunar month 11, commencing at 0657 h on 28 October 1924, and concluding at 1715 h on 26 November 1924 (see Fig. 2): −

5[A̲,B̲] → 2[A] → 3[A̲,B] → [A] → 3[A,B̲] →[A̲,B̲] → 5[A̲,B] → 3[A] → [A̲,B] → [A] → 4[A,B̲]

Lunar month 7, commencing at 2306 h on 9 July 1926, and concluding at 1348 h on 8 August 1926 (see Fig. 32): −

[...,B] →[A̲,B̲] → 6[A̲,B] → [A] → 2[A,B̲] → [A] → 2[A,B̲] →4[A̲,B̲] →4[A̲,B] → [A] →[A,B̲] → 2[A] → [A̲,B] → 3[A̲,B] → [A,...]

On days when one of the two peaks of the Etide double peak [A,B] is the major peak, its letter is underlined, either [A̲,B] or [A,B̲]. When both peaks are equal, both letters are underlined [A̲,B̲]. The incomplete entries [...,B] and [A,...] indicate that only one of the pair of [A,B] Etide peaks is present at the start and finish of the solar day.

Because of their coincidence, or near coincidence, with a singular Etide **A** peaks, the Quarter Moon phases seem to be disruptive accompaniments to the rhythm of leaf movement. Then, as either FM or NM approaches, there is a remodelling of the Etide structure. This leads to a corresponding change of the leaftide/Etide phase relationship, represented by the 'in phase' or 'in antiphase' relationships of the two oscillating tidal systems. In the case of the marine tides (Appendix Fig. A1), it is the composite [A,B] peaks designated as [A], which are disruptive of this tidal rhythm and which alter the phase relationship with the Etide.

The potential of a leaf to change the direction of its movement appears to be most keenly expressed when the rate of movement δL is zero. When this state coincides with an Etide turning point, and $\delta(\delta g) = 0$, a change of direction of the leaf can then be initiated. A series of daily turning points of leaf movement is initiated in conjunction with the new sequence of

potential Etide turning points which accompany one or other (or both) of the daily [A,B] Etide maxima and minima. Thus, $\delta(\delta g) = 0$ appears to be the threshold and/or trigger for the change of direction of a leaf movement. Whether there is further interaction between $\delta(\delta g)$ and δL lying outside the $\delta(\delta g)$ threshold range for a turning point of the movement, remains to be seen. The changing structure of the Etide throughout the lunar month allows new coincidences between $\delta(\delta g)$ and δL to be established and accounts thereby for abrupt, yet temporary, changes in the leaftide period (e.g., from 24 h to 32 h) mentioned in the appropriate section (see also Fig. 6).

In the example shown in Fig 4, of leaf movements at Akureyri, Iceland, the leaftide/Etide relationship is 'in antiphase'. However, at this location near the Arctic circle, the Etides for this lunar month, are structured with symmetrical, monotidal [A] peaks only (these are probably composite [A,B] peaks), except for one day at both FQ and LQ Moon, when the [A] peak is asymmetrical, the component Etide maxima [A,B] having different strengths (i.e., the peaks are either [A,<u>B</u>] or [<u>A</u>,B]). No change in phase relationship between leaf movements and Etide would be anticipated within this lunar month because the Etidal structure is mainly unimodal rather than bimodal. The complete structure of the Etides estimated at Akureyri for the lunar month of interest is:

Lunar month 6, commencing at 0617 h on 21 June 1925 (see Fig. 4): −

[...,B] →7[A] → [**A**] → [A,<u>B</u>] → 11[A] → [<u>A</u>,B] → [**A**] → 5[A] → 2[<u>A</u>,<u>B</u>]→[A,...].

However, the Etide structure at Akureyri during the next lunar month 7 is different and therefore might lead to a different relationship between Etide and leaftide because now there are days when the Etide is bimodal, with [A,B] peaks.

Lunar month 7, commencing at 2140 h on 20 July 1925, the Etide structure is: −

[...,B] → 2[A̲,B̲] → 5[A̲,B] → [A] → 6[A] → 2[A̲,B̲] → 4[A,B̲] →[A] → [A,B̲] → 5[A] →

[A̲,B] → [A,...].

Generally, it is difficult to predict precisely what the Etide structure at a given location will

be during any lunar month because it is affected by numerous variables (Longman 1959).

Identical Etides occur only on dates 19 y, or 6,940 d, apart (the Metonic cycle), when Sun,

Earth and Moon have identical positions relative to one another. But despite the variation of

Etides from day to day and month to month, the bean leaves maintain remarkable diurnal

consistency of their movements.

SUPPLEMENTARY DATA S6

Lunar timekeeping and leaf movements

If the rhythm of leaf movement were strictly coordinated with the rhythm of the Etide, then

leaftide rhythms would advance during the course of the month, as do the marine tides (see

Appendix Fig. A1). For example, if the Etide and leftide were continually 'in phase', then, as

well as high leaftides (i.e., leaves in the 'up' position) being present during the daytime

together with high Etides with [A,B] peaks, high Etides and high leaftides could also be

present during the night. Although, high Etides are often present during the night, for the

leaves it is clearly advantageous if they are in the 'down' (sleep) position at this time.

Contrariwise, low Etides during the daytime could be concurrent with leaves in their 'down'

position. These morpho-physiological mis-matches of the leaf are corrected by the ability of

the leaftide/Etide system to switch to the 'in antiphase' relationship whereby leaves are

enabled to achieve the 'up' position during the day, even at times when the Etide is

minimum; and, likewise, leaves can achieve the 'down' position during the night when Etide

is maximum. It is the development of the singular **A** peak during two periods of each lunar month that allows the phase relation of leaftide and Etide to be reset. The resetting comes about because, after a brief period of uncertainty (see Fig. 1 in main text), the leaf is captured by the next available Etide turning point. In this way, ascended leaves are permitted during hours of daylight and, hence, to achieve their physiological efficiency.

However, this is not the only solution to the problem of adapting the leaf position to the Etide and time of day. Resetting of the leaf tide is also assisted by the passage of the leaf through the light and dark phases, as one solar day succeeds another. This is evident not only from the effect that artificial prolongation or curtailment of either the light or dark phase has on the leaftide, as shown by Kleinhoonte (1929), but also from the consequences of light flashes given during a prolonged night period, as demonstrated by Bűnning (1954) and Bűnning and Stern (1930). The relationships between light and dark and the diurnal leaf movements are complex (Bűnning 1979) and beyond the scope of the present review but, as additional analysis has shown (PWB unpublished), light appears to sensitise the leaf to a turning of the Etide. This enables a change in the direction of leaf movement to be effected and a new relationship of the leaftide to be set with respect, not only to the Etide, but to the prevailing portion of the light/dark cycle.

That the leaf movements are able to switch from being 'in phase' with the Etide, to being 'in antiphase', and then can switch back again to being 'in phase', suggests that it is the Etide turning points per se that are influential, irrespective of whether a maximum or minimum δg value is associated with $\delta(\delta g) = 0$. It may be, also, that a range of $\delta(\delta g)$ rate values, from $\delta(\delta g) = 0$ to a $\pm\delta(\delta g)$ threshold boundary, sets the threshold which initiates either an ascent or a descent of a leaf (see, e.g., main text Fig. 4). It is as though the Etide turning point interacts with a physiological system contained within a particularly sensitive set of cells in the leaf (its pulvinus, say) which can process the tidal accelerative force to bring about a change in

that system and thereby initiate a new direction of leaf movement. The leaf system performs like a sand clock (see Discussion and Fig. 29 in main text): when a leaf is fully in the 'up' position the only movement that can follow is one that is downwards; and, conversely, when the leaf is 'down' the only available direction of movement is upwards.

SUPPLEMENTARY DATA S7

Experimental work with excised leaves

Brouwer (1926) proposed that by working with an excised leaf one is brought closer to locating the tissue responsible for processing the stimulus that affects leaf movement. Thanks to Brouwer's work, this tissue is known to reside in the pulvinus, and requires neither the participation of the shoot system, nor that of the root system with its attendant diurnal variations of root pressure.

Metabolic inhibitors

It is easy to supply the leaf, via the cut petiole, with chemicals which affect leaf movement and by so doing discover indications of the type of physiological processes involved in maintaining the leaftide rhythm. For example, interesting results were obtained by Bŭnning (1956), who used various metabolic inhibitors, by Keller (1960) working with narcotics, and by Mayer et al. (1985) using osmotic agents and membrane permeabilisers. In some cases, notably in the work of Keller (1960), the rhythms were abolished, but in other cases (e.g., after application of ethanol and methanol) the rhythmic movements of the treated leaves, following a prolongation of the first cycle by >30 per cent, became 90° out-of-phase with the rhythm of the untreated control leaves, and maintained this new relationship for five days. Often, the rhythms continued with diminished amplitude but with unaffected period. Leaves

with disturbed rhythms are susceptible to Etide and can establishing new relationships with it; hence, oscillatory movements can be maintained. Mayer (1981) repeated and extended some of the earlier work on the effects of metabolic inhibitors, and by so doing was able to identify energy-requiring phases within the leaf-movement cycle, thus clarifying Bŭnning's proposal that within the cycle there were phases of 'relaxation' and 'tension' (Bŭnning 1958, 1973).

Energy requirements

The energy requirements for leaf movement, and the regulation of energy in time and space – that is, between the upper and lower parts of the pulvinus during the movement cycle – are evidently complex. Some of this energy (the part which would have been affected by the metabolic inhibitors used in the experiments of Keller (1960) and Mayer (1981)), would have relevance for the maintenance of a sufficiency of those proteins essential to ion and water inflows and outflows. Proteins turn over fairly rapidly in growing tissues; half-lives of a few hours are typical. If pulvinar mechano-sensing proteins and ion and water transporter proteins are typical in this respect, then their energy-dependent rates of renewal during the course of a 24-h leaf-movement cycle could be considerable.

The leaf pulvinus can, moreover, be considered to be an organelle with a high potential for growth but which, at the same time, is prevented from any significant extension due to a non-extensible core provided by the vascular bundle. An analogous, though not so severe, modulation of elongation growth was proposed for the root by its stele (Barlow, 1989). Alternate phases of expansion and contraction of the flexor and extensor zones during the leaf-movement cycle (Mayer *et al.*, 1985) would require metabolism of cell wall components; in particular, a net synthesis of wall materials during their expansion phase and a net disassembly of wall during their contraction phase. The two phases of metabolism may also involve the alternating release of ions (e.g., K^+) from the wall itself; on its release from the

wall, K^+ could then serve as a regulator of osmotic pressure and, hence, of turgor pressure, and also, by its re-sequestration within the wall, K^+ could thereby serve as one of the wall's structural components (Amano *et al*., 2013). Of interest is that vanadate ions, which abolish circadian leaf movement in both *Albizzia julibrissin* and *Ph. vulgaris* (Saxe and Satter 1979; Saxe and Rajagopal, 1981), were found by Amano et al. (2013) to prevent K^+ leakage from cell walls.

A minituarised excised leaf system

A miniaturised version of the excised leaf system was developed by Yokoyama et al. (1968) for *Ph. vulgaris*, as it had been years earlier by Bűnning (1934) for *Ph. coccineus.* It consisted of a 2-cm segment of petiole, pulvinus and leaf mid-rib (the latter portion being all that remained of the leaf after removal of the lamina). When placed in an free-running environment of continuous light, and supported solely by water or a mineral ion solution, Yokoyama et al. (1968) found that, although the amplitude of movement of the mid-rib was diminished (as compared with that of control, intact leaves), the rhythm of its movement was unaffected over a span of 24-28 d, even though by this time a callus, from which roots subsequently emerged, had formed at the base of the segment. The rhythmic movements of the pulvinus of this miniature excised leaf system under continuous illumination had periods of $\tau = 28.0 \pm 0.5$ h, similar to the periods of the leaves of intact plants (Hoshizaki and Hamner 1964).

The miniaturised articulated leaf system was developed with a view to being used in microgravity experiments in space laboratories (Space Shuttle, or the International Space Station – ISS). However, complete plants of *Arabidopsis thaliana*, with their small size and non-articulated diurnally moving leaves, now seems to have superceded the miniaturised

pulvinus system (Solheim et al., 2009). The Arabidopsis system within ISS has provided strong evidence that the altered period of leaf movement in this environment conforms to the altered relationship of the ISS with the Moon. The leaf movements are tracking the changing gravitational force of the Moon during each orbit of ISS (Fisahn *et al.*, 2015).

Ageing of leaves

An interesting observation concerns the mean period of movement of primary leaves of *Ph. coccineus*, which had been raised in natural light of LD 9 : 15 and then excised and their petioles placed in distilled water under free-running conditions of constant light and temperature. When primary leaves were excised from progressively older plants and tested at weekly intervals during 40 d, it was found that their movement period declined steadily as the plant and its primary pulvinus aged; the period of older leaves was about 2.5 h shorter than that of younger leaves (Mayer et al. 1999). Moreover, primary leaves of intact soil-grown plants grown alongside the experimental group under similar constant conditions also showed this trend towards shorter movement periods as the plant aged. Periods of intact leaves were about 1 h longer than those of the excised leaves. In both cases, the greatest reduction of period occurred in the first week of the experimental period. This ageing effect remains unexplained, though evidence from the stated chlorophyll fluorescence parameters suggests that the physiological state of these plants was sub-optimal (J. Fisahn, personal communication).

In a similar experiment, but using leaves of intact plants, Allford and Tibbitts (1970) found that leaves of *Ph. angularis* showed progressively reduced amplitudes of movement during 7 d in constant light. However, the period remained constant, there being no change as the leaves aged over the 7-d growth timespan. A possible explanation for the reduced amplitude

in light is that excessive photosynthetic activity in the absence of dark-period metabolism interferes with the leaf movements (see Simon *et al*., 1976).

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