

Lunar gravity affects leaf movement of *Arabidopsis thaliana* in the International Space Station

Joachim Fisahn¹ · Emile Klingelé² · Peter Barlow³

Received: 17 December 2014 / Accepted: 13 March 2015 / Published online: 21 March 2015
© Springer-Verlag Berlin Heidelberg 2015

Abstract

Main conclusion Cyclic leaf ascent and descent occur in synchrony and phase congruence with the lunisolar tidal force under a broad range of conditions.

Digitized records of the vertical leaf movements of *Arabidopsis thaliana* were collected under space flight conditions in the International Space Station (ISS). Oscillations of leaf movements with periods of 45 and 90 min were found under light-adapted conditions, whereas in darkness, the periods were 45, 90, and 135 min. To demonstrate the close relationship between these oscillations and cyclical variations of the lunisolar gravitational force, we estimated the oscillations of the in-orbit lunisolar tide as they apply to the ISS, with the aid of the Etide software application. In general, in-orbit lunisolar gravitational profiles exhibited a periodicity of 45 min. Alignment of these in-orbit oscillations with the oscillations of *Arabidopsis* leaf movement revealed high degrees of synchrony and a congruence of phase. These data corroborate previous results which suggested a correlative relationship and a possible causal link between leaf movement rhythms obtained on ground and the rhythmic variation of the lunisolar tidal force.

Keywords Circadian clock · Etide · Lunisolar Zeitgeber

Abbreviations

ISS International Space Station
EMCS European Modular Cultivation System

Introduction

Since the time of some of the earliest natural philosophers (e.g., Pliny the elder, 23–79 AD), the movements displayed by plant organs have held a continuing fascination (Sweeney 1969) and numerous studies have aimed to demonstrate their correlation with geophysical parameters. One of the first documented attempts to elucidate whether the rhythm of movement was inherent to a plant or was the result of external stimuli was that of de Mairan (1729). He observed that the rhythmic leaf movements of *Mimosa pudica* continued even during extended periods of darkness (de Mairan 1729), and that they were also affected by environmental cues. Over the years that followed, researchers increasingly attributed rhythmic leaf movements to an endogenous circadian clock. Although certain patterns of leaf movement produced results inconsistent with a regulator, or *Zeitgeber*, that was purely endogenous (Flügel 1949), gene-expression networks could be established as an ‘explanation’ for the diurnal regulation of many biological rhythms, including those of leaf movements (Harmer 2009). Moreover, two or more distinct rhythms, which affect different life processes, can be contained within one and the same cell (Roenneberg and Morse 1993), organ (Hennessey and Field 1992), or plant (Rascher et al. 2001; Lüttge 2003), and each being regulated by a different

✉ Joachim Fisahn
fisahn@mpimp-golm.mpg.de

¹ Max Planck Institute of Molecular Plant Physiology,
Am Mühlenberg 1, 14476 Potsdam, Germany

² Institute of Geodesy and Photogrammetry,
ETH-Hönggerberg, 8093 Zurich, Switzerland

³ School of Biological Sciences, University of Bristol,
Bristol Life Sciences Building, 24 Tyndalls Avenue,
Bristol BS8 1TQ, UK

timekeeper. Therefore, it seems possible that any of the one or more controversial actuators of leaf movement could operate simultaneously and thereby provide two or more complementary timekeeping signals (Barlow and Fisahn 2012).

Due to the availability of new geophysical methodology, leaf movements and certain other phenomena in plants have been demonstrated to be coupled to the lunisolar gravitational force (Barlow and Fisahn 2012). This force is the result of the rotation of the Earth around its axis in conjunction with the orbital motions of the Earth and its Moon around the Sun. As a consequence, this force continually modulates the gravitational field of the Earth (Konopliv et al. 1998, 2001), an effect that is most pronounced and readily measurable in relation not only to the tidal movements of seas and oceans but also to small variable and elastic deformations of the Earth's crust. These latter are measurable by gravimetry (Xu et al. 2004; Crossley et al. 2005). In particular, the recently developed 'Etide' program enables local variations of the Earthly gravitational acceleration, δg , due to the lunisolar tidal force, at past and future times to be estimated (Longman 1959; Barlow and Fisahn 2012). These estimates, therefore, provide an opportunity to search for synchronized variations within biological and geophysical time courses. The idea that the unforced leaf movements of bean plants might be regulated by the lunisolar tidal force was introduced by Barlow et al. (2008; see also Klein 2007). Examples of the proposed relationship between these two variables, one biological and the other geophysical, could be established for leaves of the bean plant, *Canavalia ensiformis*, which were subject to two different sets of natural conditions (Barlow and Fisahn 2012). The conclusion drawn from these and other observations on bean leaves of various species is that any one of the sequence of 'turning points' in the lunisolar gravity profile, when the rate of change of the gravitational acceleration is zero, is the condition that alters the direction of leaf movements and, hence, the periodicity of these movements. In addition, attention was drawn to an apparent concordance between the daily variation in tree stem diameter, δD , and the variation in the gravimetric tide, δg (Zürcher et al. 1998; Barlow et al. 2010); and, similarly, with the aid of the Etide program, Fisahn et al. (2012) observed that roots of *Arabidopsis thaliana* appeared to perceive the lunisolar tidal acceleration, this perception being expressed in a tide-like variation in the rate of root elongation. In short, although a large body of evidence has been collected for the interaction of the lunisolar gravitational force and plant growth and behavior, this evidence is so far entirely correlative. A more direct establishment of the concept would require the ability to modify, in some defined way, the lunar gravitational force and then detect the effects of this modification

on leaf movement rhythms which hitherto had been considered mainly to be subject to regulation by a putative endogenous *Zeitgeber*, or timekeeper.

Although it is not possible to alter the lunisolar gravitational force experienced by living organisms on Earth, it is possible to predict how this lunisolar influence would vary outside the confines of the Earth. For example, within the extra-terrestrial orbit of the International Space Station (ISS), the rhythm of the lunisolar tidal force would be quite different to any rhythm that it has on Earth. This prediction requires not only a consideration of the orbital parameters of the ISS but also an ability to estimate the lunisolar gravitational force within the ISS itself. It follows that, due to these two factors, rhythms of leaf movement which develop within the ISS should be different from those displayed on Earth in terms of their period. Moreover, because the gravitational pull of the Earth is strongly reduced at the location of the ISS, the gravitational acceleration of the Moon would be perceived without a strong terrestrial gravitation background. In addition, the location of the ISS with respect to the Moon varies constantly during every orbit around the Earth, resulting in corresponding fluctuations of the gravitational forces, as perceived within the ISS. The ISS orbits the Earth approx every 90 min and, hence, rhythms within the ISS, with two high and two low lunisolar tides per orbit, would be expected to lie within the ultradian range rather than the circadian. Indeed, ultradian movements of *A. thaliana* rosette leaves were discovered and studied under microgravity conditions in space (Solheim et al. 2009). These ultradian oscillations were characterized by periods of 45, 90 and 135 min (Solheim et al. 2009). In the present study, we shall test whether the periodicities and phase relations of these detected ultradian rhythms in leaf movement are compatible with, and supportive of, the concept that the experimental plants within the ISS are responding, as evidenced by the rhythm of their leaf movements, to the lunisolar gravitational accelerative force, and that this interpretation is entirely sufficient and does not require any adaptation or innovation of a putative endogenous timekeeping mechanism.

Materials and methods

The experimental results, revisited here, were collected during the first long-term experiment in the European Modular Cultivation System (EMCS) within the framework of the experiment "MULTIGEN-1" (MULTIple GENerations 1) (Solheim et al. 2009; Johnsson et al. 2009). The EMCS was located within the US Destiny module of the ISS. The design of both the EMCS and the experimental containers has been described (Brinckmann 2005; Fossum et al. 2005; Solheim et al. 2006).

Plant material

Seeds of *A. thaliana* (L.) Heyhn., ecotype Columbia, which originated at Lehle Seeds, Round Rock, TX, USA, were transported to the ISS and were germinated 16 days later. Plantlets were transferred to experimental containers within the EMCS. Further development took place within a regime of white light and darkness (LD 16:8 h) (Solheim et al. 2009; Johnsson et al. 2009) and was recorded on video tape. Temperature was held constant at 23 ± 0.5 °C (Brinckmann and Brillouet 2000). Constant humidity was automatically maintained. Each movable video camera within the EMCS collected images simultaneously from 2 experimental containers, and 4 video cameras collected images from the full on-board complement of 8 experimental containers. Each experimental container harbored one plant cultivation chamber with three or five seed holes. The complete setup provided 32 holes for seed germination. Each seed hole contained one to several seeds, depending on the experimental objective. All leaves of the developing rosettes of *A. thaliana* could be monitored adequately by a newly developed video equipment (Solheim et al. 2009). Therefore, reliable time courses of leaf movement could be digitized and committed to a data base for future analysis.

When the *Arabidopsis* plantlets were around 42 days old, their leaves exhibited several types of movements (Solheim et al. 2009). Vertical movements of 3 leaves on each plant were simultaneously recorded by video and were found to be in phase. Hence, in each experiment, movements of a single reference leaf from one plant were taken as representative for all leaves of that particular plant. During the 16:8-h light and dark periods, all vertical leaf movements were recorded by infrared video imaging and the trajectories of the leaf tips digitized. The relevant time courses of these movements were analyzed using Fast Fourier transform (FFT), as already described by Solheim et al. (2009).

Determination of ISS orbital coordinates and on-board lunar gravity profiles

The hourly positions of the ISS during its orbit are available from data bases provided by NASA at: <http://spotthestation.nasa.gov/sightings/#.U1jMq9KSySE>; and <http://spaceflight.nasa.gov/realdata/tracking/index.html>. These positions can then be projected orthogonally to Earth and read as Earthly longitudes and latitudes (Fig. 1b). An estimate of the lunisolar gravitational acceleration within the ISS can be obtained at each time and at each projected location using the Etide program. The Etide program is based upon the 50 parameters which compute the vertical component of the lunisolar tidal force (the horizontal component of which is negligible) (Longman 1959). The input to Etide, in addition to latitude and longitude, consists

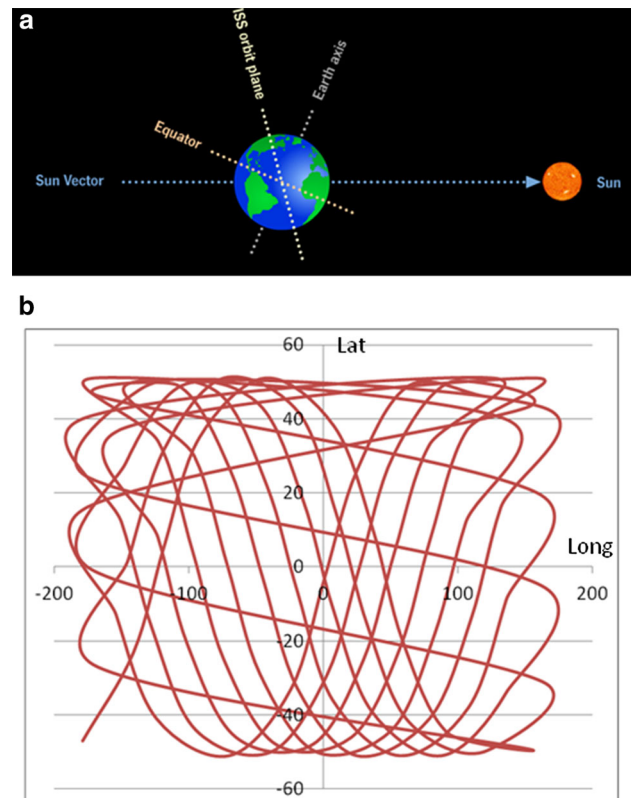


Fig. 1 Determination of the ISS orbital parameters. **a** Schematic representation of the relative orientation of the Earth's axis and the ISS orbit (angle β). **b** Geographical coordinates (latitude and longitude orthogonally projected to the Earth's surface) of the ISS orbit on 8th of October 2007 between 0600 and 2200 hours (compare Solheim et al. 2009, upper trace, their Fig. 3)

of the altitude of the location in question (ISS orbit), together with the calendar dates for which local tidal estimates are required. The output is a time course of δg (a gravity profile) which increases and decreases with respect to the orbital location of the ISS and the relative positions of the Sun and the Moon. With the aid of the Etide program, biological data can be analyzed retrospectively for putative lunisolar relationships, the only requirement being that the numerical biological data are linked to a particular location and, crucially, to a particular calendar date. Timeframes denoted in the present study refer to Greenwich Mean Time (UTC).

Drift elimination

To eliminate the slow change in growth kinetics during the period of leaf movement observations, a sixth-order polynomial was subtracted from the experimentally obtained results (Solheim et al. 2009). The linearized leaf movement timecourse was then aligned with the estimated timecourse of the lunisolar gravity profile derived from the Etide software.

Results

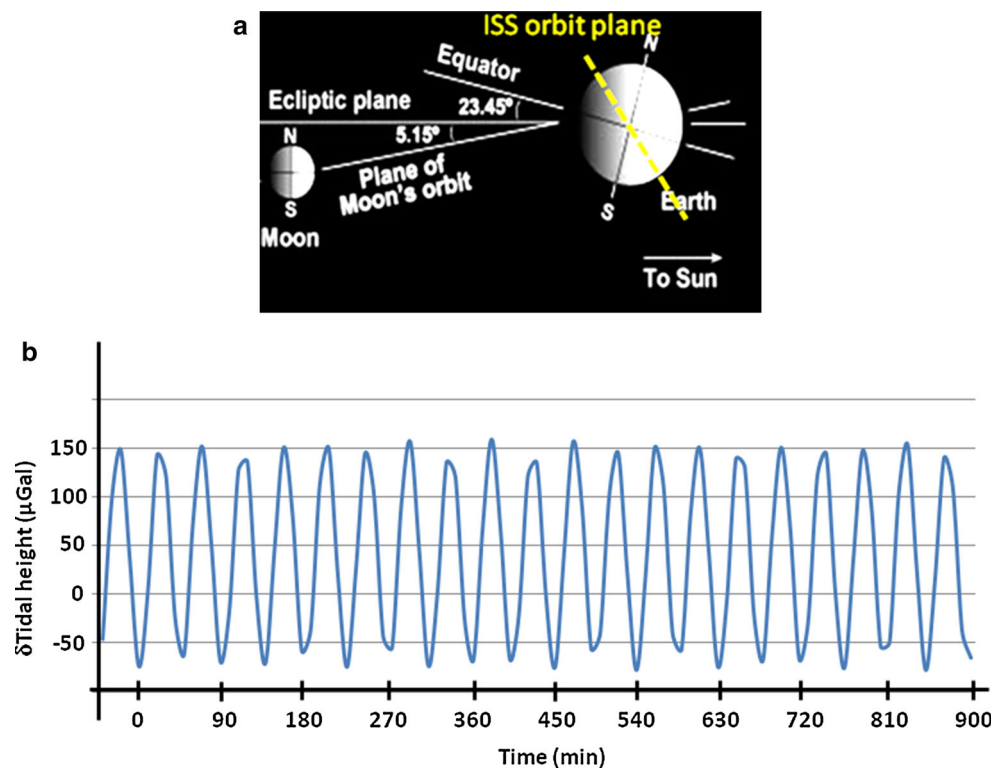
Determination of the geographical coordinates of the ISS orbit

To align leaf movement records and tidal profiles experienced by the ISS, the orbital coordinates of the ISS were independently determined for each individual experiment. As the ISS completes 15.410 orbits around the Earth per day, the period τ of one orbit amounts to approximately 90 min. Further orbital details of the ISS are depicted in Fig. 1a. Because the Earth rotates around an axis different from that of the ISS, the start of each ISS orbit, in terms of Earthly longitude, is advanced relative to the starting position of the previous orbit cycle (Fig. 1a). Thus, within the 16-h light phase of the experiment analyzed, the ISS traverses a broad range of geographical coordinates (Fig. 1b). Moreover, due to the varying angle between the Earth's axis and the orbital axis of the ISS, and the difference in their respective orbital times of 24 h and 90 min, there is no periodicity of traverse, by the ISS, of the same geographical coordinates. The geographical position of the ISS (Fig. 1b) has, therefore, to be calculated for every experimental time point.

Determination of the lunisolar gravitational force experienced by the ISS

Due to the rotation of the Earth around its axis and the gravitational forces exerted by the moon, marine tides at locations on Earth occur with a periodicity of 24.8 h. The marine tide may be considered to be an approximate counterpart of a lunar-drive tidal force that affects the Earth. This is, in fact, the Etide. Because the ISS orbits the Earth (Figs. 1b, 2a) within a period of 90 min, two high and two low lunar tides, induced by the cyclic passage of the lunar gravitational field, should be felt within the ISS during this time period. Knowledge of the orbital coordinates permits an estimation of the lunisolar gravitational acceleration within the ISS by means of the Etide program (Barlow and Fisahn 2012). These estimates match the expectation of two high and two low lunar tides during one complete 90-min orbit of the ISS (Fig. 2b), because the ISS, in its orbit around the Earth, experiences the proximity of the Moon twice during this period. The day-by-day estimates of the lunisolar gravitational profiles were aligned with the recorded in-orbit leaf movement kinetics.

Fig. 2 The ISS orbit-associated lunisolar tidal pattern estimated by Etide. **a** Relative orientation of the lunar orbital plane and the ISS orbital plane. **b** Variations of the lunisolar tidal acceleration (δg) in accordance with the ISS orbital location depicted in Fig. 1b, estimated at 10-min intervals using the program 'Etide'. The units of δg are expressed in μGals . The gal, sometimes called galileo, (symbol *Gal*) is a unit of acceleration used extensively in the science of gravimetry. The Gal is defined as 1 cm per second squared (1 cm/s^2). 1 Gal is equal to 0.01 m/s^2



Alignment of *Arabidopsis* leaf movement cycles with the in-orbit ISS lunar tidal profiles during the light period

During the 16-h light period on board the ISS, two types of leaf movement oscillations emerged that were characterized by periods of 45 and 90 min. When the timecourses of these oscillations were aligned with the timecourses of the estimated in-orbit lunisolar gravitational profile, an unequivocal coincidence became apparent between the two parameters (Fig. 3). In particular, the 45-min oscillation of leaf ascent and descent was exactly in phase with the 45-min oscillation of the estimated lunisolar tidal profile (Fig. 3a). However, commencement of the 45-min leaf oscillation was preceded by a period of insensitivity to the lunisolar gravitational acceleration (Fig. 3a). Similar periods of insensitivity in leaf movements to the lunisolar gravitational force have been described for leaves investigated in Earth-bound laboratories (Barlow and Fisahn 2012), and these have been related to periods of relaxation and tension during the leaf movement cycle (Mayer 1981). Commencement of leaf ascent coincided with an increase in the lunisolar tidal acceleration. Then, upon attaining sensitivity to the lunisolar acceleration, the cycle of the

reference leaf immediately became synchronized with the cycle of the in-orbit lunisolar gravitational profile for the next 5 complete periods.

In addition to the 45-min rhythmicity of *Arabidopsis* leaf movement during the conditions of space flight in ISS, a period doubling—that is, a 90-min series of ultradian oscillations—was reported to prevail during the 16-h light period (Solheim et al. 2009; Fig. 3b). The 90-min periodicity was preceded by a weak 45-min oscillation of leaf ascent/descent, as shown in Fig. 3b. These 90-min cycling kinetics of the *Arabidopsis* leaves were also aligned with the estimated in-orbit lunisolar gravity profiles (Fig. 3b). In this portion of the experiment, the lunisolar profile showed several strongly reduced maxima and, hence, this portion of the profile was less regular (Fig. 3b, blue line). Similar modulations in the amplitude of the lunisolar gravitational peaks occur during each month at many geographical locations on the Earth; they reflect the relative positions of the Earth, Moon and Sun, i.e. the lunar phase on the dates of interest (Barlow and Fisahn 2012; Fisahn et al. 2012). During this period of 90-min periodicity of leaf cycling, every second ascending slope in the lunisolar gravity profile was concordant with a leaf movement (Fig. 3b). Although the main oscillation in leaf cycling in the second part of the timecourse exhibited a 90-min periodicity, the 45-min period was nevertheless still weakly superimposed upon the general pattern of leaf movement (Fig. 3b). A similar response to every second peak in the lunisolar gravity profile has been described for leaf movement patterns in Earth-bound observations (Barlow and Fisahn 2012). However, in contrast to the ISS results, with periodicities of 45 and 90 min, the terrestrial leaf cycling oscillations show periods of 12.4 and 24.8 h (Barlow and Fisahn 2012); this being largely due to the timing of Moonrise and Moonset.

The maximal values (peaks) in the lunisolar gravitational profiles are not necessarily required for triggering the 90-min oscillation of leaf ascent/descent movements under space flight conditions. Figure 3c demonstrates that this 90-min oscillation can be also triggered by highly symmetrical lunisolar gravity profiles. In this situation, every second rise in the in-orbit Etide profile appears to force an ascent of the reference leaf. However, the peak which was ignored appears able to affect the slope of the leaf movement rhythm. Therefore, leaf descents were slower than leaf ascents. This finding suggests that an asymmetry exists in the mechanisms of leaf ascent and descent. A similar asymmetry of ascent and descent under laboratory conditions on Earth was reported for *Arabidopsis* leaves by Engelmann et al. (1992) and for *Phaseolus* leaves by Mayer (1981). Recovery of a more constant phase relationship between the lunisolar and leaf oscillations again demonstrated a putative link between the geophysical and

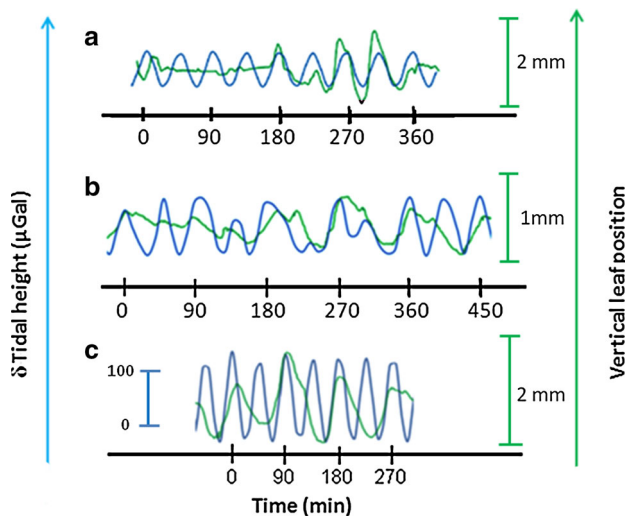


Fig. 3 Alignment of leaf movement kinetics (green line) for three different *Arabidopsis* plants with the in-orbit lunisolar gravity profiles (blue line) during exposure to continuous white light in the EMCS. **a** The reference leaf exhibits a 45-min oscillation of its movements which is in synchrony with the in-orbit lunisolar gravitational profile. **b** An initial 45-min oscillation is followed by a 90-min oscillation of leaf movements. **c** A 90-min oscillation of leaf movement which is in phase with the lunisolar gravitation profile. Every second peak of the lunisolar gravitational profile was ignored by the oscillating leaf. However, every second leaf ascent positively correlates with the ascending lunar tidal profile. Overall, an asymmetry in the time constants of leaf ascent and descent emerges. Each leaf movement trace in a–c (green line) represents three independent leaves moving in synchrony

biological parameters. Furthermore, it should be remarked that the experiment in Fig. 3c was performed on developmentally older leaves than in the previous examples. Therefore, some type of developmental switch might account for the above-mentioned doubling of the leaf movement period.

Together, we suggest that ultradian leaf movement rhythms of *A. thaliana* in the light-adapted conditions are induced by the continually varying lunisolar gravitational force experienced within the ISS during its orbit around the Earth.

Alignment of *Arabidopsis* leaf cycles with ISS in-orbit tidal profiles during the in-orbit dark period

Ultradian oscillations of leaf movement under space flight conditions were detected during the in-orbit dark period (Solheim et al. 2009). These oscillations exhibited periodicities of 135 min, 90 min and, to a minor extent, 45 min (Fig. 4).

During the in-orbit dark period, the initial ascent of the in-orbit lunisolar gravitational force is synchronized with the ascending movements of the reference leaf (Fig. 4a). As described above, the subsequent leaf descent occurred more slowly than the previous ascent; and it is noticeable that two Etide peaks which follow are ignored by the descending

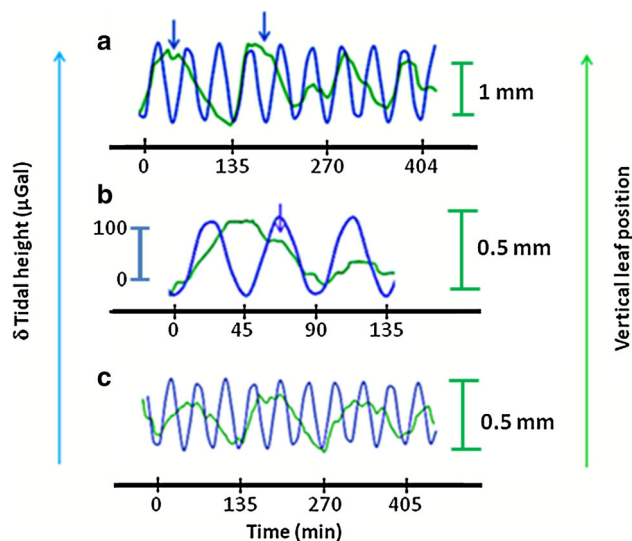


Fig. 4 Ultradian oscillations in *Arabidopsis* leaf movements (green lines) under dark-adapted conditions within the ISS aligned with the in-orbit lunisolar gravity profiles (blue lines). **a** Three distinct periods in leaf ascent/descent occurred during the dark portion of the experiment: 135, 90, and 45 min. All three periods were detected within one dark period. **b** A second example of the occurrence of 90-min and 45-min oscillations of leaf cycling. However, in this case, oscillations in leaf ascent/descent did not persist throughout the entire dark period. **c** In a third example, leaf movement kinetics were time-aligned with the inverted lunisolar gravitational profile under dark-adapted conditions. Each leaf movement trace in **a–c** (green lines) represents three independent leaves moving in synchrony

leaf. Synchrony is regained during the second ascent of the leaf, exactly following the fourth rise of the lunisolar tide. Similar to the initial leaf descent, the immediately following minimum (trough) of the lunisolar gravity coincided with the next descent of the leaf and induced a small transient indent in the kinetics of the descent (arrows in Fig. 4a). The occurrence of these weak intermediate troughs (arrowed in Fig. 4a) in the leaf movement record indicates that an unknown mechanism inhibited the sensitivity of the leaf during its descent and thus disallowed the leaf from following exactly the variation in lunisolar gravity. The second descent of the leaf proceeded at a slightly faster rate than the first descent. Consequently, an intermediate peak in the rate of leaf movement occurred that exactly coincided with the sixth peak of the lunisolar gravitational profile (Fig. 4a). Despite this intermediate local maximum of leaf movement, the next major peak of movement coincided with the seventh peak of the lunisolar gravity profile. At the end of the dark period, the predominant 135-min periodicity in leaf cycling was reduced to 90 min (Fig. 4a). In summary, if a stimulus which triggers an ascent, which in this case was a rise in the tidal force, is received by the plant, leaf cells thereby become excited and a movement response is initiated. During the execution of this movement, the excitation of the cells is checked, and in this state they become insensitive to further signals. Thus, there is a refractory period during which the next incoming signal cannot be processed.

Not all plants showed identical leaf movement patterns during the dark period. Figure 4b depicts a further example of leaf movement cycling derived from another *Arabidopsis* plant, from a second experiment. Leaf ascent was triggered by increasing lunisolar acceleration, but during the subsequent refractory period, the next anticipated leaf descent is checked and one peak of the in-orbit lunisolar tidal profile was ignored. However, this missed lunisolar peak nevertheless slightly affected the phase relationship of leaf movement with Etide, inducing a plateau in the slow relaxation kinetics of the leaf descent (Fig. 4b; arrow). On the basis of the approximately 45-min periodicity of the in-orbit lunisolar gravity profiles, ultradian rhythms in leaf movement with periods of 45, 90 and approximately 135 min are all evidently multiples of the coincidental 45-min lunisolar tidal periodicity.

A special phase correlation between leaf movement and the in-orbit lunisolar gravitational profile is depicted in Fig. 4c. The leaf cycling rhythm is here characterized by an approximately 135-min periodicity. However, the alignment of lunisolar tidal profile and leaf movement depicted in Fig. 4c was performed with an inverted tidal profile. Similar synchronizations between leaf movement rhythms and inverted lunisolar profiles have been described elsewhere, for plants grown in laboratories on Earth (Barlow and Fisahn 2012; Fisahn et al. 2012). Reasons for this

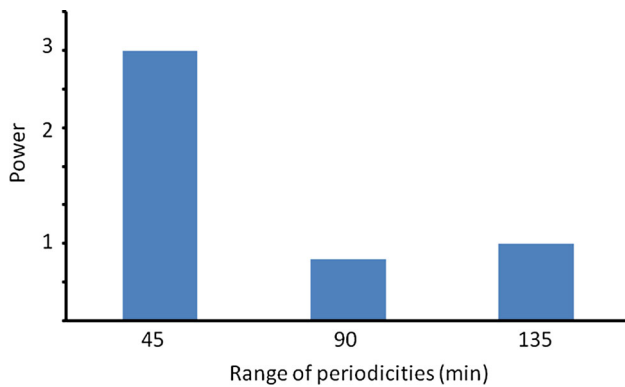


Fig. 5 Statistical distribution of oscillations in leaf movement characterized by periodicity ranges. The data represent the recording of one typical leaf followed over 9 light periods, according to Solheim et al. (2009, their Fig. 6). As a result, the 45-min oscillation was the predominant rhythm detected in all leaves recorded. In detail, the period content in the microgravity “hot pixel” data of Solheim et al. (2009) was analyzed by the fast Fourier transform technique. Periods and pulsed rhythms in the 45 min region were always apparent

inverse type of correlation can presently only be speculated upon. One hypothesis attributes this type of coincidence to an unknown second force that could interfere with the lunisolar gravitational force in inducing either leaf ascent or descent. Whether this force implies a physical origin related to plant developmental or nutritional aspects remains to be elucidated. However, as pointed out elsewhere (Klein 2007; Barlow and Fisahn 2012), it is likely that it is the turning point per se which is the critical determinant of a change in leaf movement direction, irrespective of whether this is associated with a peak or a trough of lunisolar tidal variation. Overall, the sum of the two putative forces would be to drive water into the cells mediating leaf ascent during a decrease in lunisolar gravitational force.

According to Solheim et al. (2009), the 45-min oscillations were continuously present in all leaves investigated on board the ISS (Fig. 5). Overall, 24 leaves could be distinguished by the newly developed software application (“hot pixel”) and their movements were followed over several days, or even weeks (Solheim et al. 2009). The typical distribution of the three major periodicities is depicted in Fig. 5. Thus, the frequency of occurrence of the 45-min oscillations strongly exceeded that of either the 90-min or the 135-min oscillation (Fig. 5).

Discussion

Comparisons between the oscillations of leaf movements of *A. thaliana* plantlets grown in the ISS and those of the in-orbit lunisolar gravitational profiles have revealed high degrees of synchrony (Figs. 3, 4). This synchrony was predicted from the results obtained from leaf movement

analyses performed in Earth-bound laboratories (Barlow and Fisahn 2012), where a pronounced correlation emerged between the peaks and troughs of the oscillating lunisolar tidal force and the periodic 24.8-h oscillations of leaf movement cycles of various species of bean plants (Barlow and Fisahn 2012). The experimental confirmation of such a highly reproducible coincidence between the periodicity of a geophysical and of a biological parameter provides a definite indication of a putative causal relationship between them. To substantiate the impact of the lunisolar tide on leaf-associated rhythms, Barlow and Fisahn (2012) analyzed the phase relations between these two parameters. At all locations, and for all plant species tested, an unequivocal concordance of phase gave rise to the suggestion of a close interrelationship between leaf movement and lunisolar acceleration for plants grown on Earth (Barlow and Fisahn 2012). Further circumstantial evidence for this close correlation, which even implies causality, as found on the Earth, was demonstrated in the present study of space-grown plants within the ISS. Because the ISS orbits the Earth more rapidly than the Earth rotates upon its axis (Fig. 1a, b), lunar gravity-associated “circadian” rhythms of leaf movement within the ISS exhibit periodicities in the ultradian range, of approximately 45 min or multiples thereof (Figs. 2a, b, 3, 4). The phase relationship between the lunisolar gravitational profiles in the ISS and the timecourses of the ultradian leaf movements were compared and found to coincide (Figs. 3, 4). Therefore, this additional evidence, collected in-orbit for the existence of an explanatory correlate between leaf movement and lunisolar gravity, corroborates our previous conclusions from on-ground-derived data.

Many of the leaf movement experiments on Earth were performed under free-running conditions of either continuous light or dark. The selected light and dark periods within the ISS were 16 and 8 h. Although these durations have physiological relevance to plant growth on Earth, in the ISS, because of its 90-min orbit around the Earth, the light and dark periods within the EMCS, because they are far longer than the periods of the leaf movements, provide free-running conditions for the study of leaf movement rhythms (Figs. 3, 4). Thus, because the light and dark periods were of lengths that were perceived as continuous during the course of the observations, entrainment signals due to the EMCS light/dark regime may be ruled out as a trigger of the ultradian oscillations of leaf movement. It is concluded that these movements are exclusively determined by the frequency with which the lunisolar gravity field varies during each orbit of the ISS.

The leaf movements recorded in Figs. 3 and 4 occurred on different plants and on different calendar dates within the ISS. Although the recurring LD 16:8 h periods within the EMCS mimic the daily light/dark cycles on Earth, they

do not hold a fixed phase relation with the variation of lunisolar gravitation force experienced either on Earth or in the ISS. In particular, commencement of each dark or light period within the ISS happened to coincide with a changing phase in the in-orbit lunisolar gravitational profile. But even so, the phase congruence between leaf movement rhythms and the in-orbit lunisolar gravity profile was maintained in every experimental recording (Figs. 3, 4). Substantial evidence is, therefore, obtained for an explanatory correlation between the biological and the geophysical parameter on a statistical basis. Hence, a purely accidental congruence in the aligned parameters can be discounted. Moreover, 45-min oscillations in leaf ascent and descent were detected in all leaves investigated (Fig. 5). 45-min rhythmicity coincides with half of the 90-min orbital periodicity of the ISS. Therefore, the 45-min oscillations are not directly linked to one full cycle of the ISS, but to a causal event with a doubling of its periodicity. As outlined in the results section, two high and low lunisolar tides emerge during one complete rotation of the ISS giving rise to a frequency doubling and thus producing 45 min rhythmicity, as predicted. Due to the 45-min periodicity in tidal recurrence on-orbit, the amplitudes of the cyclic leaf movements are strongly reduced (Figs. 3, 4) in comparison to the 24.8 h amplitudes recorded for Earth-bound locations.

Although the exact mechanisms of the movement of *A. thaliana* leaves are not known yet, the oscillatory ascending and descending movements of the leaves are mediated in part by a respective alternating influx and efflux of water into and out of particular cells located on the abaxial and adaxial portions of leaf epidermis of *Arabidopsis* (Uehlein and Kaldenhoff 2008; Rauf et al. 2013). Slower oscillations with a periodicity of 120 min occurred only during the dark period (Fig. 4), indicating that water movement within the leaf probably decreased during darkness. This diminution of flux might have been a consequence of the stomatal closure during the dark period. In particular, evaporation of water vapor through the stomatal pore is strongly inhibited in the dark, thus forcing a slower descent of the leaf. A similar mechanism of stomatal aperture variation could have set the periodicity of the leaf movement cycle in the light-adapted situation.

It cannot be discounted that the lunisolar tide also influences or regulates the rhythm of stomatal opening and closure. It is interesting, therefore, that Lasceve et al. (1997) demonstrated that the stomatal conductance of *A. thaliana* continuously increased and decreased during the light period even under conditions of constant illumination in a growth chamber. However, it is not known whether stomatal aperture or conductance is modulated either by the microgravity environment of ISS or by lunisolar gravity; and if the latter, whether this effect is overturned by cycles

of light and dark. The *Arabidopsis elf3* (early flowering 3) mutant shows arrhythmic leaf movement under conditions of continuous light (Dornbusch et al. 2014). Consistent with an effect of stomatal aperture on leaf movement, it was demonstrated that water relations in this mutant are strongly affected by their inability to close their stomata (Kinoshita et al. 2011). *Zeitlupe* (*ZTL*) mutants of *A. thaliana* exhibit a 32-h periodicity in both leaf cycling (Somers et al. 2000) and stomatal conductance (Dodd et al. 2004) under conditions of continuous light. Moreover, the effect of *ztl-1* on period length is strongly dependent on the photon flux densities (Somers et al. 2000). Therefore, the different periods of the circadian oscillations in stomatal conductance and CO₂ assimilation rate in *ztl-1* could be interpreted as a consequence of the different light intensities being received by the circadian oscillators of the guard cells and the mesophyll cells (Dodd et al. 2004). In *ztl-1*, the predicted consequence is that the free-running period of circadian rhythms of stomatal movements on the lower surface will be longer than the period of both the rhythms of stomatal movements on the upper surface and the CO₂ assimilation rate (Dodd et al. 2004). For soybean plants, the leaflet angle was linearly correlated with stomatal conductance and photosynthetic rate suggesting a close association between leaf orientation and leaf metabolism (Rosa et al. 1991). A close relationship between stomatal aperture and leaf movement is not restricted to herbaceous plants, but is also observed in the tropical tree species, *Acacia aroma* (Hernandez and Arambarri 2010).

The mechanisms involved in the perception and processing of the lunisolar field strength by plants are neither understood nor identified at present, even though the physiology of leaf movement is quite well known (see Moran 2007). Nevertheless, quantum gravitational models have been developed which could account for the described causal relationship between the lunisolar gravity profile and the leaf movement rhythms (Dorda 2010).

The transcriptome of *A. thaliana* was compared under space flight and ground control conditions (Paul et al. 2013). 480 genes exhibited significant changes in expression in space flight plants compared to ground control. However, the range of genes affected by spaceflight appears to spread beyond those genes whose expression might be easily explained by changes in gravity, and include a number of genes with roles in the response to a wide variety of environmental factors (Paul et al. 2012). A pronounced induction of transcripts mediating a response to drought stress emerged under space flight conditions (Paul et al. 2012). In particular, when plants were grown in the ISS, transcripts At3g08770 (LTP6), At3g55740 (PROT2), At1g04110 (SDD1) which are transcripts that are known to be induced upon drought stress, exhibited a 16.8-, 6.96-, and 5.9-fold induction, respectively (Paul et al.

2012). It has to be pointed out that all plants were automatically watered within the EMCS, similar to the ground controls, and that the latter control plants did not show any symptoms or transcriptomes that would suggest that plants in the ISS growth setup suffered drought stress. Perhaps, the drought stress relates to the rapid cycling of stomata aperture between open and closed. Therefore, there is no evidence that the leaf movements are a response to cycles of water availability. It is concluded, therefore, that the experiments performed in the ISS provide evidence that the described movement patterns of *A. thaliana* rosette leaves are caused solely by the lunisolar tidal force.

Author contribution JF and PB designed the research and wrote the ms. JF performed the calculations. EK provided a new version of the Etide program suited for the ISS orbits.

Acknowledgments We are grateful to Prof. Dr. Anders Johnsson (Department of Physics, Norwegian University of Science and Technology, N-7491 Trondheim, Norway), Dr. Bjarte Solheim (Department of Physics, Norwegian University of Science and Technology, N-7491 Trondheim, Norway), and Prof. Dr. Tor-Henning Iversen (Department of Biology, The Plant BioCentre, Norwegian University of Science and Technology, N-7491 Trondheim, Norway) for providing additional results and details of the conditions in the ISS during the in-orbit experiments, for reading the manuscript and providing valuable comments. We would like to thank Prof. Dr. Nima Yazdanbakhsh for critical discussion of the described experiments. Special thanks to Prof. Dr. em. Enno Brinckmann for providing valuable further details on the construction of the EMCS.

Conflict of interest We declare no conflict of interests.

References

- Barlow P, Fisahn J (2012) Lunisolar tidal force and the growth of plant roots, and some other of its effects on plant movements. *Ann Bot* 110:301–318
- Barlow PW, Klingelé E, Klein G, Mikulecký M Sr (2008) Leaf movements of bean plants and lunar gravity. *Plant Signal Behav* 3:1083–1090
- Barlow PW, Mikulecký M Sr, Střeščík J (2010) Tree-stem diameter fluctuates with the lunar tides and perhaps with geomagnetic activity. *Protoplasma* 247:25–43
- Brinckmann E (2005) ESA hardware for plant research on the International Space Station. *Adv Space Res* 36:1162–1166
- Brinckmann E, Brillouet C (2000) Space plant research on the ISS with the European Modular Cultivation System and with BIOLAB. SAE Tech Pap Ser Paper No. 2000-01-2472
- Crossley D, Hinderer J, Boy JP (2005) Time variations of the European gravity field from superconducting gravimeters. *Geophys J Int* 161:257–264
- de Mairan J (1729) Observation botanique. *Hist Acad Roy Sci (Paris)* 1729:35–36
- Dodd AN, Parkinson K, Webb AAR (2004) Independent circadian regulation of assimilation and stomatal conductance in the *ztl-1* mutant of *Arabidopsis*. *New Phytol* 162:63–70
- Dorda G (2010) Quantisierte Zeit und die Vereinheitlichung von Gravitation und Elektromagnetismus. Cuvillier Verlag, Göttingen Germany. ISBN-10: 3869552409. <http://www.cuvillier.de/flycms/de/html/30/-UickI3zKPS7xcE0=/Buchdetails.html>
- Dornbusch T, Michaud O, Xenarios I, Fankhauser C (2014) Differentially phased leaf growth and movements in *Arabidopsis* depend on coordinated circadian and light regulation. *Plant Cell* 26:3911–3921
- Engelmann W, Simon K, Phen CJ (1992) Leaf movement rhythm in *Arabidopsis thaliana*. *Z Naturforsch* 47:925–928
- Fisahn J, Yazdanbakhsh N, Klingelé E, Barlow PW (2012) *Arabidopsis thaliana* root growth kinetics and lunisolar tidal acceleration. *New Phytol* 195:346–355
- Flügel A (1949) Die Gesetzmässigkeiten der endogenen Tagesrhythmik. *Planta* 37:337–375
- Fossum K, Kittang AI, Iversen T-H, Brinckmann E, Schiller P (2005) Testing the European Modular Cultivation System (EMCS) for ISS plant and cell research. In: International conference on environmental systems, Rome, ITALY. SAE Technical Paper Series 2005-01-2841
- Harmer SL (2009) The circadian system in higher plants. *Annu Rev Plant Biol* 60:357–377
- Hennessey TL, Field CB (1992) Evidence of multiple circadian oscillators in bean plants. *J Biol Rhyth* 7:105–113
- Hernandez MP, Arambarri AM (2010) Stomatal distribution, stomatal density and daily leaf movement in *Acacia aroma* (Leguminosae). *Bol Soc Argent Bot* 45:273–284
- Johnsson A, Solheim BGB, Iversen T-H (2009) Gravity amplifies and microgravity decreases circumnutations in *Arabidopsis thaliana* stems: results from a space experiment. *New Phytol* 182:621–629
- Kinoshita T, Ono N, Hayashi Y, Morimoto S, Nakamura S, Soda M, Kato Y, Ohnishi M, Nakano T, Inoue S, Shimazaki K (2011) *FLOWERING LOCUS T* regulates stomatal opening. *Curr Biol* 21:1232–1238
- Klein G (2007) Farewell to the internal clock. A contribution in the field of chronobiology. Springer, New York
- Konopliv AS, Binder AB, Hood LL, Kucinkas AB, Sjogren L, Williams JG (1998) Improved gravity field of the moon from Lunar Prospector. *Science* 281:1476–1480
- Konopliv AS, Asmar SW, Carranza E, Sjogren WL, Yuan DN (2001) Recent gravity models as a result of the Lunar Prospector mission. *Icarus* 150:1–18
- Lasceve G, Leymarie J, Vavasseur A (1997) Alterations in light-induced stomatal opening in a starch deficient mutant of *Arabidopsis thaliana* L. deficient in chloroplast phosphoglucosyltransferase activity. *Plant, Cell Environ* 20:350–358
- Longman IM (1959) Formulas for computing the tidal accelerations due to the moon and the sun. *J Geophys Res* 64:2351–2355
- Lüttge U (2003) Circadian rhythmicity: Is the “biological clock” hardware or software? *Prog Bot* 64:277–319
- Mayer W-E (1981) Energy-dependent phases of the circadian clock and the clock-controlled leaf movements of *Phaseolus coccineus* L. *Planta* 152:292–301
- Moran N (2007) Osmoregulation of leaf motor cells. *FEBS Lett* 581:2337–2347
- Paul AL, Zupanska AK, Ostrow DT, Zhang Y, Sun Y, Li JL, Shanker S, Farmerie WG, Amalfitano CF, Ferl RJ (2012) Spaceflight transcriptomes: unique responses to a novel environment. *Astrobiology* 12:40–56
- Paul AL, Zupanska AK, Schultz ER, Ferl RJ (2013) Organ-specific remodeling of the *Arabidopsis* transcriptome in response to spaceflight. *BMC Plant Biol* 13:112
- Rascher U, Hütt MT, Siebke K, Osmond B, Beck F, Lüttge U (2001) Spatiotemporal variation of metabolism in a plant circadian rhythm: the biological clock as an assembly of coupled individual oscillators. *Proc Nat Acad Sci USA* 98:11801–11805

- Rauf M, Arif M, Fisahn J, Xue G-P, Balazadeh S, Mueller-Roeber B (2013) NAC transcription factor speedy hyponastic growth regulates flooding-induced leaf movement in *Arabidopsis*. *Plant Cell* 25:4941–4955
- Roenneberg T, Morse D (1993) Two circadian oscillators in one cell. *Nature* 362:362–364
- Rosa LM, Dillenburg LR, Irwin N, Forseth IN (1991) Responses of soybean leaf angle, photosynthesis and stomatal conductance to leaf and soil water potential. *Ann Bot* 67:51–58
- Solheim BGB, Kittang AI, Iversen T-H, Johnsson A (2006) Preparatory experiments for long-term observation of *Arabidopsis* circumnutations in microgravity. *Acta Astronaut* 59:46–53
- Solheim BGB, Johnsson A, Iversen T-H (2009) Ultradian rhythms in *Arabidopsis thaliana* leaves in microgravity. *New Phytol* 183:1043–1052
- Somers DE, Schultz TF, Milnamow M, Kay SA (2000) *ZEITLUPE* encodes a novel clock-associated PAS protein from *Arabidopsis*. *Cell* 101:319–329
- Sweeney BM (1969) Rhythmic phenomena in plants. Academic Press, London
- Uehlein N, Kaldenhoff R (2008) Aquaporins and plant leaf movements. *Ann Bot* 101:1–4
- Xu J, Sun H, Ducarme BA (2004) A global experimental model for gravity tides of the Earth. *J Geodynamics* 38:293–306
- Zürcher E, Cantiani M-G, Sorbetti Guerri F, Michel D (1998) Tree stem diameters fluctuate with tide. *Nature* 392:665–666