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Peter Barlow's insights and contributions to the study of tidal gravity variations and ultra-weak light emissions in plants

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Research in Context

Title:

Peter Barlow's insights and contributions to the study of tidal gravity variations and ultra-weak light emissions in plants

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Running headings:

Cristiano M Gallep et al. — *Peter Barlow's insights to the study of tidal gravity (...)*

• *Background:* Brief review of Peter W Barlows' contributions to research on gravity tide-related phenomena in plant biology, or 'selenonastic' effects as he called them, including his early research on root growth. Also, new results are presented here from long-term recordings of spontaneous ultra-weak light emission during germination, reinforcing the relationship between local lunisolar tidal acceleration and seedlings growth.

• *Scope:* The main ideas and broad relevance of the work by Peter Barlow and his collaborators about the effects of gravity on plants are reviewed, highlighting the necessity of new models to explain the apparent synchronism between root growth and microscale gravity changes 10^7 times lower than that exerted by the Earth's gravity. The new results, showing for the first time the germination of coffee beans in sequential tests over two months, confirm the co-variation between the patterns in ultra-weak light emission and the lunisolar tidal gravity curves for the initial growth phase. For young sprouts (less than one month old), the rhythm of growth as well as variation in light emission exhibit the once a day and twice a day periodic variations, frequency components that are the hallmark of local lunisolar gravimetric tides. Although present, this pattern is less pronounced in coffee beans older than a month.

• *Conclusions:* The apparent co-variation between ultra-weak light emission and growth pattern in coffee seedlings and the lunisolar gravity cycles corroborate those previously found in seedlings from other species. It is proposed here that such patterns may attenuate with time for older sprouts with slow development. These data suggest that new models considering both intra- and intercellular interactions are needed to explain the putative sensing and reaction of seedlings to the variations in the gravimetric tide. Here, a possible model is presented based on supracellular matrix interconnections.

Key words: germination, lunisolar gravity tide, ultra-weak light emission

INTRODUCTION

" Gravity is a uniform background presence during development; it has clearly played a role in shaping the course of plant and animal evolution, and biological constructions are now in harmony with the force that gravity imposes."

Peter W Barlow (2007)

Peter W Barlow's statement constitutes the panoramic window through which the circadian rhythmic behaviour of plants and animals alike can now be scrutinized and meaningfully interpreted. The universality of this vision will be the guiding light of this tribute to a remarkably insightful scholar.

Lunar rhythms are traditionally used by communities all over the world as a tool to assert the best germination and harvest time. This attention to the phase of the Moon is hailed to promote the best yield of the final product, including both quantity and quality of the harvested food or wood (Kollerstrom and Staudenmaier 2001, Zürcher 2001).

In his typically forensic and critical way, Peter Barlow explored published data related to such 'Moon-phase' phenomena, and also helped many groups in exploring new biological data that appeared somehow related to the local gravimetric oscillations, δg, occurring daily as result of the Sun and Moon action (referred to as the lunisolar cycle here) over the Earth's surface gravity. This data mining united and contextualised a wealth of diverse cyclical phenomena such as, leaf movements, tree stem diameter and electric potential (EP), stem growth and nutation, root growth, and also spontaneous ultra-weak photon emission (UPE) from sprouts. This contribution will focus on UPEs.

The study of intriguing effects of cyclical lunar phase on plant biology arose only recently amongst Peter Barlow's multiple interests. Among those are ground-breaking forays into the mathematical modelling of plant development presenting novel ideas uniting cell morphology to plant organisation and root architecture, or exploring the role of the cytoskeleton in wood cell development in trees (Chaffey 2017). During his professional employment and also well into retirement, Peter Barlow demonstrated an enduring and enviable ability to explore new territories, on his own, with own resources, or in collaboration with other scientists embracing similar fascination for less trodden yet fundamental aspects of biology. Those who had the good fortune to work and debate with Peter will recognise his boundless generosity and passion for scientific exploration.

To understand the pathway Peter Barlow constructed to advance the study of gravimetric tide effects on plant biology, we need to consider some of his earlier cell and root studies, mainly those pertaining to rhythms, movement and growth.

Barlow discussed how tropic movements in plants would occur in an intricate chart proposed in 1992, trying to elucidate the ways plants would react to external factors, including consideration to gravity (Barlow 1992). A detailed discussion on plant morphogenesis was then developed in a further substantial article, taking rhythm, periodicity and polarity as the most general properties of living matter:

> "[shoot and root growth] depends primarily upon rhythmic switches in the polarity of cell growth. These rhythms coincide with, and may even be dependent upon, unequal potentialities of daughter cells following a quantal mitosis or some supracellular quantal event in the apex."
>
> (Barlow 1994, emphasis ours)

What could be such 'supracellular event'? The question remains open, but we shall get back to it later.

Peter Barlow's specific interest in the oscillatory movements of plants while growing appears in an article of 1994 (Barlow *et al.* 1994). There, he describes and discusses the minor and major movements of roots, with a special focus on the putative physical information and possible detection threshold involved (Barlow *et al.* 1994). In a hallmark twist, he also contends that such movements are the observable, objective expression of the natural presence of external physical drivers – to which humans may be naturally insensitive (Barlow *et al.* 1994). This vision is reminiscent of what is collectively known as the sensory ecology approach (Dusenbery 1992), and how the physical organisation of nature affects the lives of organisms in space and time (the physical ecology approach).

Along the path of discovery, Barlow contributes further insights and a deep discussion on gravity perception as a sensing capacity that is phylogenetically ancient in plants, and that may probably exhibit today a rich functional diversity (Barlow 1995). There, this important assertion can be found:

> "Thus, primitive mechanisms of gravisensing may now coexist with others which arose independently at later stages of evolution. The discovery of old and new mechanisms together would give the impression that gravisensing is a process with in-built redundancy, although this would not be true if different graviperception systems have distinct roles at particular stages of development."
>
> (Barlow 1995)

The different gravisensing mechanisms that might exist in a single plant would act towards one common, integrated response, perhaps in redundancy, or act as distinct and complementary mechanisms, each one of them serving the detection of distinct physical quantities. In that work, a flow-chart is also provided for plants and 'lower' organisms that addresses the increase in complexity of the putative detection systems, from those based on statoliths (structures using the migration of dense intracellular calcite or starch granules to sense gravity), that would fit for bacteria and algae, to those based on nucleus displacement, fit for fungi, and finally to amyloplasts (intracellular starch granules within statocytes), as seen in higher plants (Barlow 1995).

While Barlow was providing advice for experiments to be conducted on the International Space Station, he discussed the impact of microgravity on plants, pointing to possible developmental disturbances, with insights that may help to resolve the question of the putative ‘supracellular’ mechanisms:

> “Many gravity responses of organisms (their tropisms and taxes) may trace to boundaries, such as the plasma membrane, where mass (acceleration) would be intercepted. The properties of the boundary that follow from interception of acceleration (gravisusception) may then serve as preconditions for further ‘events’, and so on. (…) <u>It would be interesting if, given the range of possibilities in normal development, as exhibited by plasticity, to discover whether gravity supplies any unique preconditions for the accomplishment of a developmental event at either the molecular or morphological levels</u>.”
>
> (Barlow 1998, emphasis ours)

It was also clear to Barlow that root cap dynamics were key to understand how gravity acts on growth. He later advanced this point further, stating:

> “(…) there are intriguing suggestions of some kind of physiological link between the border cells surrounding the cap and mitotic activity in the cap meristem. Open questions concern the structure and functional interrelationships between the root and the cap which surmounts it, and also the means by which the cap transduces the environmental signals that are of critical importance for the growth of the individual roots, and collectively for the shaping of the root system“.
>
> (Barlow 2002)

The rhythmic aspect of plant development in space was developed together with Jacqueline Lück (Barlow and Lück 2008), where it was stressed that “the repetitiveness of large-scale branching events, as well as the smaller scale of repeated idioblastic cell development, are related to the rigorous clock-like mechanism which governs cell reproduction". This realisation in effect established the need to better understand the temporal (rhythmic) aspects of morphogenesis, a theme that is thereafter omnipresent in Barlow's approach to plant development and its plasticity and this will be evident later.

Peter Barlow's first study on the relation of leaf movements to the local gravimetric tide appeared in 2008 (Barlow *et al.* 2008), where he carefully developed a meta-analysis of Klein's data (Klein 2007). Barlow and collaborators showed that the nastic movement of leaf blades appears synchronous to the local gravimetric changes due to the relative movement of Sun and Moon. Numerous examples, from different species and cultivars, indicated that an increasing tidal force usually depresses the leaf downwards, and that rapid leaf bending movements occurs when there is a local change in the tidal microgravity δg, ie when such " force changed from either a minimum ('low tide') or a maximum ('high tide')" (Barlow *et al.* 2008). In keeping with Peter Barlow's efforts to spread news concepts and ideas, he suggested a name for gravity tide-related phenomena: 'selenonastic' effects (from the Greek Selene = Moon) (Barlow *et al.* 2008).

The similarity between δg time patterns and the diameter variation of tree stems were later explored with collaborators (Barlow *et al.* 2010). Exploring published data from seven species of tree at two different locations growing in natural and in controlled conditions, this article proceeded to examine tree stem oscillation patterns in view of local gravity fluctuations. While mindful of the distinction between causation and correlation, these papers concluded that the lunar component of the gravity variation alone could influence stem diameter variation and that, "under certain circumstances, additional regulation may come from the geomagnetic flux" as well. (Barlow *et al.* 2010).

At that moment in time, Peter Barlow felt that solid specific empirical data were needed and, now retired, he secured key collaborations, renewing his efforts to collect crucial data. Importantly, the co-variation with local gravimetric tide was also found for the growth of *Arabidopsis thaliana* roots (Fisahn *et al.* 2012, Barlow and Fisahn 2012, Barlow *et al.* 2013). Using accurate video recording of root tips in controlled conditions, growth velocity was traced in time, and growth found to be in phase with the δg profile. The data show common periodic components between δg and the rate of root elongation. In that study, the irregular natural variations of the solar geomagnetic flux reaching the Earth was also considered, and proposed as a possible additional factor acting over growth cycles. Although geomagnetic storms are quite rare and unpredictable, and difficult to avoid in normal laboratory conditions, they are known to be correlated with Sun and Moon positioning in relation to Earth, akin to the δg tide. In effect, disentangling the lunisolar tidal effects from those relating to variations in the geomagnetic flux is considered quite difficult to determine in standard laboratory conditions (Barlow *et al.* 2013).

In 2012, Barlow developed further the idea of gravity tide as an extrinsic "developmental modulator" of life processes in a book chapter (Barlow 2012). There, he hypothesised that the adaptive value of the mechanisms at work for gravity sensing are also related to the bio-availability of water – a molecule evidently crucial and ubiquitous for germination and growth. In this work, Barlow mined historical data of bio-electric potential (EP) from trees collected by different groups in the 1940's, 90's and 2000's. The diverse data available show that the daily EP cycle is co-variant with the δg tide, and that their respective amplitudes are related in a linear way. Monthly time-resolved data clearly exemplified the co-occurrence of EP oscillations and δg during an entire Moon cycle. EP fluctuations were also shown to be proportional to water content in the tree, rather than to transpiration rate. Altogether a link was proposed between water content and bio-availability, and the gravimetric tide (Barlow 2012).

The hypothesis that the local gravimetric tide is acting over leaf movements was supported by results of tests run in the International Space Station (ISS) (Fisahn *et al.* 2015) –

during its 90-min. orbit around the Earth the ISS undergoes two complete tidal cycles and leafs presented cyclic ascent and descent with 45-min and 90-min period, occurring in synchrony and phase congruence with the lunisolar tidal force, even for different illumination conditions.

A review on leaf movements and their relationship with the lunisolar gravitational force appeared in 2015 (Barlow 2015), including many new plots for different species where leaf changes appears co-variant to δg cycles. The increasingly abundant supportive data permitted Barlow to develop ever more detailed hypotheses, suggesting once again that:

> "a lunisolar clock, in which the *zeitgeber* is exogenous and independent of metabolism would lie in a category of 'primal' biological phenomena that could allow both animal and plant organisms to continue to express rhythmic patterns of behaviour under conditions where light is absent".
>
> (Barlow 2015)

The last of his contribution on seedlings' movement appeared only recently, showing that the stem growth, the nutation and the leaf movement in peppermint (*Mentha piperita L.*) also follow local δg cycles, corroborating the enticing proposition of a gravimetric tidal *zeitgeber* (Zajazczkowska and Barlow 2017).

How far are we now from understanding the causes and mechanisms of variation of growth in plants, and how complete is our current phenomenological description? Enticingly, another, previously elusive, physical parameter has attracted some attention in the past decade: the ultra-weak photon emission (UPE) occurring in growing seedlings and their relation to δg cycles.

Ultra-weak photon emission is understood to be a consequence of radiative decay (luminescence) of electronically excited states of molecules which are continuously generated in metabolically active organisms (Cifra and Pospíšil, 2014). The mechanism underlying the generation of excited states and consequent UPE is currently understood to be as follows: metabolism, and its associated oxygen consumption, which takes place mainly in mitochondria, chloroplasts, peroxisomes, endoplasmic reticulum, but also in cell wall bound oxidases, membrane NADPH oxidases and apoplasts (Møller 2001, del Rio 2015, Das and Roychoudhury, 2014), leads to the production of reactive oxygen species (ROS). Reactions of ROS with a wide range of lipids, proteins and nucleic acids can produce high energy intermediate molecules: dioxetanes (Bastos et al. 2017) and tetroxides (Miyamoto et al. 2007). These molecules can decompose to produce either excited species such as triplet excited carbonyl (Bastos et al. 2017) or singlet oxygen (Miyamoto et al. 2007), which can, in turn, directly emit photons or, after incurring some delay, transfer excited state energy to acceptor molecules (Cifra and Pospíšil, 2014). These acceptors can then also emit photons (Cifra and Pospíšil, 2014). This description pertains only to the direct pathway which leads to production of UPE. However, at every step described above, there are many other competing pathways which do not lead to UPE.

The UPE from sprouts during growth was discovered some time ago by Colli *et al.* (1955) and is generally understood as a manifestation of metabolic activity during germination and early seedling growth (Rafieiolhosseini et al. 2016) and has been used as real-time, non-invasive diagnostic probe of vigour (Gallep 2014). UPE was also found to be related to lipid peroxidation (Havaux et al. 2006) and to ROS activity under flooding stress (Kamal and Komatsu 2015) in plants.

The first study reporting cycles in spontaneous UPE from seedlings in relation to the local gravimetric tide benefited from generous help and support from Peter Barlow (Moraes *et al.* 2012). The evidence gathered shows that seedlings' growth is accompanied by UPE, and that the UPE signal present temporal variations similar to that of local δg, exhibiting coincident turning points and related periodic components. Analysis of long term time series of consecutive germination tests over a duration of about 2 months, during which UPE were recorded for the 2nd and 3rd days of germination of wheat (*Triticum aestivum L.*), showed also that both UPE intensity and sprout's elongation vary in a similar way during the lunar month. Further, the UPE for a single sunflower seedling also appears in co-variation to the local δg (Gallep 2014).

Because geolocalisation determines the shape of δg varation, experiments were also conducted at two different locations in parallel, e.g. in Neuss, Germany, using local and transported samples, and in Limeira, Brazil, using local samples (Gallep *et al.* 2013). Testing variations of UPE as a function of different δg input, the data from that study reveal that the UPE of both local samples have profiles with similar periodic components (as assessed using Fourier transforms) to those of the local gravimetric tide (~12.2h and 24.4h). Noteworthy is the occurrence of many coincident inflection points in time. Interestingly, the UPE profile of the seeds transported from BR to DE presented time profiles with reduced periodicity, lacking coincident inflection points with local gravity tide. In effect, Fourier analysis reveals periodic components distinct from those of the main δg cycles, exhibiting some beat frequencies and additional harmonics (around 6h, 15h and 18h). It must be noted that the days when the seeds were transported by air (São Paulo-Neuss) coincided with a period of strong geomagnetic disturbance related to Sunspot activity, a composition of circumstances difficult to replicate that may have influenced seed development.

A second test with transported seeds was run with support of labs in Japan (Hamamatsu city) and Czechia (Prague), in September 2012 (Gallep *et al.* 2014). UPE of wheat germination tests run simultaneously in these two localities and in Limeira/BR all did show co-variation with its local gravimetric profile. Notably, tests conducted in Japan used samples transported from Brazil one week earlier and did not show abnormal UPE variation. The same seed stock was brought back to Brazil and measured in parallel and simultaneously to not-transported, resident samples; both presented similar UPE profile, with regular turnings points coincident with those of the local gravimetric variation (Gallep *et al.* 2014).

A further contribution in this field appeared recently, in collaboration with colleagues at the University of Leiden, Netherlands (Gallep *et al.* 2017). UPE were measured from wheat seedlings taken from a stock transported overseas from Brazil to the Netherlands (direct São Paulo-Amsterdam) and back, in July 2014, taken in parallel with those from local stocks. In this case, strong UPE-gravimetric correlations were measured for the local samples, as expected, and for the sample transported from NL back to BR. Similarly to the tests conducted earlier in Neuss, the samples tested in Leiden that had been transported from Limeira lacked the periodicity of the other samples. A linear relation between the local amplitude of δg and of UPE was present for both local and transported samples, indicating that both disturbed (transported) and resident samples do respond more strongly to gravity tide cycles with larger magnitudes. It was also shown that the UPE profiles for seedlings of different species measured in parallel, e.g. wheat-corn and corn-sunflower (Helianthus annuus), present turning-points consistently coincident with those of the local gravity tide (Gallep *et al.* 2017).

Here, we present new results of UPE for coffee seedlings, taken during the whole germination period of 2 months. Owing to their slow germination, coffee seedlings enable long-term recordings, which have revealed the presence of slower variations not found before for sprouts with faster germination: as seedlings mature, the periodic components of the gravimetric tide become gradually less pronounced, unveiling a slower periodicity, with a period of ca. 4 days.

MATERIALS AND METHODS

Coffee germination

Coffee beans (*Coffea Arabica*), selected to discard damaged beans at the Federal University of Lavras (UFLA - MG/BR), were put to germinate at 05-Feb-2016 using standard method with water-saturated filter paper rolls vertically arranged over plastic containing trays. This stock of seedlings were moved in optimal conditions to U.Campinas/Limeira (SP/BR) on 06-Feb-16 and kept inside germination chambers, in the dark, with controlled constant humidity (70% ± 10%) and temperature (32°C ± 2°C) for the next two months. This is the usual germination time for coffee. Eight filter paper rolls containing 50 seedlings each were maintained through February and March/16, from where samples were collected every week for the photon-count tests.

Photon-Count tests of seedlings

Photon-count (PC) measurements of the spontaneous light emission were run for seedlings samples chosen from the stock. Starting on 10-Feb-16, every week thereafter, a sample of seedlings was selected as representative of actual sprout stage and put in a petri dish, with a 10-cm diameter filter paper and 10 mL of demineralized water. To obtain a strong light signal, 12 seedling sprouts were positioned on the paper without touching each other. During February PC measurements contained 12 seedlings per run, in March it was reduced to 8 seedlings (t1) and then to 6 seedlings (t2 and t3) per run.

The transfer of seedlings from rolls to dish was done under minimal light exposure to avoid strong delayed luminescence; the dish was then put inside the dark chamber for photon-counting measurements, whereby the sample holder provided temperature control through a regulated thermal bath (set to 32°C) to optimize growth (Gallep 2014). PC acquisition started just after putting samples in dark, integrating counts in 10-s time discretization, and recorded continuously throughout the PC test (~7 days).

Each sample was photographed before and after PC measurements, to assess seedling development in comparison to the main stock.

Data analysis

Photon count data (counts/10s) of each test were smoothed, reducing signal variance by averaging adjacent 100 data points. A second-order polynomial fitting was then used to remove long term trend for each test, and the remaining oscillatory profile was again smoothed by adjacent 1000 data points averaging, in order to reveal inflection points and enable comparison with the smooth gravimetric tide profile.

Gravimetric tide calculation

A computer program (ETIDE) was used to calculate the gravimetric tide, which essentially provides an estimate of the local gravitational pull resulting from the combined actions of both Sun and Moon at a chosen location on Earth. ETIDE is based upon the 50 parameters, first used by Longman (1959), for computing the vertical gravimetric component of the lunisolar tidal force. The horizontal component was not included the present lunisolar tide computations. Briefly, the inputs to ETIDE consist of the latitude, longitude and altitude of the location in question (Limeira, SP/BR; 22° 33' 53" S & 47° 24' 06" W, 700 m high) together with the calendar dates for which estimates are required. The computational output is δg, expressed in μGals (1 g = 9.81 × 10^8 μGals), and represents the increase and decrease of the Earth's gravitational acceleration at any particular location brought about by the combined gravitational forces of the Sun and Moon.

Periodic components

To determine the frequency content of both gravimetric and PC data, periodograms were created using Welch's method (Welch, 1967) with 50% window overlap, which allows the recovery of low frequency data. Here, frequency content is given in cycles per day (1/d). Spectra were normalized such that their total power is equal to 1, facilitating comparison of relative powers at specific frequencies.

RESULTS

The PC data after initial smoothing but prior to detrending is presented in the Supplementary Material, as well as pictures taken from each sequential sample just before and after PC measurement.

The detrended PC data (d-PC) for three tests in February 2016 are presented in Figure 1, and also for 3 more tests in March 2016 (Figure 2). Data are superimposed to the local δg and its first derivative, $\delta g/\delta t$. Due to a momentary technical failure, one test at beginning of March was lost, constituting the only discontinuity in PC measurements of the two-month germination test. Test t3 in March (23d to 26d, Fig.2) has its vertical axis (d-PC) divided by 3, to normalise it to other tests, since this last sample exhibited very strong light emission (see PC data in Supplementary Material).

Inflection points of the d-PC curves are highlighted with a pair of vertical arrows, pointing to direct comparison with both time-resolved δg and $\delta g/\delta$ functions. Numerous, but not all, d-PC inflexions are coincident with variations in the local gravitational pull, with most rapid changes in d-PC coinciding with fast inflections in δg, where rate of change ($\delta g/\delta t$) is maximal.

The long-term temporal organisation of the PC data and how it relates to lunisolar cycles can also be depicted in the frequency domain (Fig. 3). The normalized PC periodograms for all six tests, three on each month, reveal the regularity of UPE. When considered along the frequency structure of the δg function, the common periodic components with the photon emissions becomes apparent (Fig. 3b). In the linear ordinate plots, main trends in amplitude variations are apparent across the dynamic range of the response, while in logarithmic scale, small variations in amplitude are highlighted, revealing the presence of low power components of the d-PC oscillations.

DISCUSSION

Coffee requires a very long germination period, of 2 months under optimal conditions for sprouts to develop both roots and leaves. This is much slower than other species tested previously, such as corn and wheat whose sprouting takes less than a week, corresponding to a quarter of a Moon cycle. In contrast, the development of coffee sprouts spans the passage of two lunar cycles. This means that the same sample of seedlings can be measured continuously along multiple cycles of the semidiurnal gravimetric oscillations. In addition, long recording times also enable the evaluation of the full range and gravimetric amplitude variations, changing from maximum to minimum four times in two months (Figs. 1, 2).

Similarly to previous work on other plant species, initial seedling growth shows numerous coincident inflection points between d-PC and δg profiles, and also coincident long term trends, with d-PC progressing very similarly to the δg curve. This is exemplified by data for time periods 12.5d to 13d, 17.4d to 18d, 24.5d to 25d, and 25.3d to 25.6d in February 2016 (Fig.1). Yet, this effect tends to diminish as the sprouts get older and the d-PC exhibits a decrease in the amplitude of the periodic components around 1/d and 2/d (Fig. 3, March). A moderate and progressively waning response is observed around 11.2d to 11.5d, on 13d to 14d, on 17.2d to 17.7d and on 25.8d to 26.2d of March 2016 (Fig.2).

Another recurrent feature in this set of data is that the coincident inflexion pairs usually alternates between δg and its first time derivative, with few exceptions noted mostly for the last test in March. These data suggest that gravimetric tide minima and maxima affect the UPE of seedlings, yet that also the rate of change of δg, its velocity, causes variations in photon emissions.

Noteworthy is that the last test (t3/March) exhibited very strong light emissions, despite the presence of only 6 seedlings, with average photon counts higher than 150/s (see Supplementary Material).

This evidence reveals that as the sprouts get older and bigger, photon counts increase, presumably accounting for the mass increase of metabolically active tissue. This is similar to UPE observed in other species (Gallep 2014). But the cycle components with frequencies around 1/d and 2/d, that were pronounced at the beginning, fade away at the end of March's tests, when longer period components ($f < 0.5$/d) contribute to the majority of the oscillatory power (Fig. 3). Components of one cycle per day are always present for the d-PC data, even when the δg component is small around that periodicity (as in t3 February). The main δg periodic component, around 2/d, appears also for d-PC data in February, with very small amplitude for t1 Mar (ca. 10^{-3}, see Fig. 3 log scale).

One possible explanation for the fact that older seedlings showed reduced UPE period components, as compared to younger seedlings, is that, as they have more developed leaves, they would be more sensitive to light. Even if minimal, exposure to light can occur when transferring samples from the growing stock to the photon-count setup. Such transfer took place once in a week. This potential exposure to light exposure could constitute a cumulative input for the older samples, possible contributing to the very low frequency UPE periodic components.

One outstanding question pertains to how small changes in local gravity can have an effect on the growth of seedlings. In effect, the amplitudes of variation of δg are estimated to be around 10^6 to 10^7 smaller than the average Earth's acceleration g. How can roots and/or leaves sense microscale variations in gravity, and the forces induced by them? This issue is related, we propose, to the so-called kT paradox whereby, in standard conditions, temperature vibrations at room temperature, or thermal motion, is at least as large as the effects δg can theoretically impart on the plant tissue. Considering the buoyancy intrinsic to cell organelles in their environment, and the estimated δg (ca. 100 μGal), it can be predicted that large bodies are needed (ca. 100 μm radius) for microgravitational pull to overcome thermal motion. This theoretical critical size (and mass) is however bigger than the conventionally accepted gravisensing organelle, the amyloplasts. At their largest, amyloplasts located in root cap statocytes have a radius of tens of μm (Hinchman and Gordon 1974). In addition, owing to medium viscosity, the gravity driven response of amyloplasts requires forces to act steadily for durations in excess of 1000 s. The present evidence reveals response times in the range of tens of seconds, introducing a discrepancy between the dynamics of amyloplast motion, UPE response and the drive by gravimetric tide (see Supplementary Material). In this context, it is worth mentioning that the gravimetric sensing considered here is not atypical of mechanical sensing in general. In effect, across all hearing animals studied, the auditory sensory organs readily sense variations in pressure, called sound by definition, that are at least 10^8 smaller than the static atmospheric pressure (Robert and Göpfert 2002).

We contend that a physically plausible mechanism for sensing microgravity forces must involve multiple amyloplasts, or other dense structures. By way of hypothesis, it may be worth considering here the proposition that these are the interactions -or concerted actions- between a collection of amyloplasts that enable, putatively, the sensing of small variations in gravity. This contention is similar to that proposed by Peter Barlow in 1995.

By analogy to active mechanisms in hearing and their role in frequency selectivity and enhanced sensitivity, an active process operating outside thermal equilibrium is hypothesised to be present in the root tip and to be sensitive to small δg perturbations. Tentatively, we posit here that mechanoreceptive molecular mechanisms may not be localized within one cell only, limited to its small collection of amyloplasts, as plant gravitropism is conventionally understood to work (Barlow 1995). We surmise that ensembles of amyloplasts mechanically connected by cytoskeletal filaments (or other, perhaps undescribed yet, structures) are involved, operating together across several adjacent cells, that is at the supracellular level. Also, speculatively, sensitivity to microscale gravity variation could involve the mesoscopic action of water, since coherently organized and mobile clusters of water could provide electrical-mechanical input to cells in response to conformational or positional changes induced by the gravimetric tide.

This supracellular hypothesis was discussed with Peter Barlow in the past years, and he had many assertive points to claim that this could be possible. Barlow and Chaffey have shown that cytoskeletal elements associated with the cell wall help during cell division, involving myosins, microtubules (MT) and microfilaments (MF) and acting cooperatively in a supracellular matrix to support growth:

> "(…) Linking the cytoskeleton-mediated long-distance symplasmic transport within the axially oriented sieve tubes/sieve cells with the radial pathway of solutes mediated by the ray cells would create a super-symplasmic continuum which would, in turn, permeate the whole tree. The role that this three-dimensional network might have for co-ordination of developmental processes remains to be explored."
>
> (Chaffey and Barlow 2002, emphasis ours)

The essential idea emerging from this line of thought and evidence is that intercellular cytoskeletal connections may constitute the substrate for sensing microscale variations in gravity – an assertion that clearly requires to be tested experimentally. Herewith, we propose a conceptual model involving a long-range network of supracellular connections across the root cap (Fig. 4). The organisation of this network is overlaid to an original picture of a corn root tip from Barlow (2003). The key proposition resides in the use of the orientation of cellular walls as the guideline for the structured network. By following the continuous lines formed by cell walls, three main types of 'lanes' can be distinguished – longitudinal, U-like and transverse. The longitudinal lanes are long, running from proximal to distal and arranged orthogonally to the U-like lanes. U-lanes are located around the proximal root cap edge (Fig. 4), extending laterally to run in parallel to the lateral lanes. Altogether, this network could act as a mechanical strain gauge sensitive in 3 directions. Mechanical sensitivity is proposed to be provided by the concerted actions of amyloplasts, acting as the inertial elements, and linked molecular components, including microfilaments and motor proteins such as myosins. This proposition is evidently speculative and hopefully can generate the impetus for the development of novel molecular genetics assays, accompanied by biomechanical work to address the molecular composition of intercellular connections, the nanoscale sensitivity of cytoplasmic filaments associated with amyloplasts, and further studies into the susceptibility of root caps to small changes of gravimetric variations.

SUPPLEMENTARY DATA

Time series for the PC data for germination tests, including second-order fit used to detrend signal growth, are presented. Pictures of each sample, before and after PC measurements are shown as well as explanation on calculations for the microgravity force over a cell organelle.

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Figures

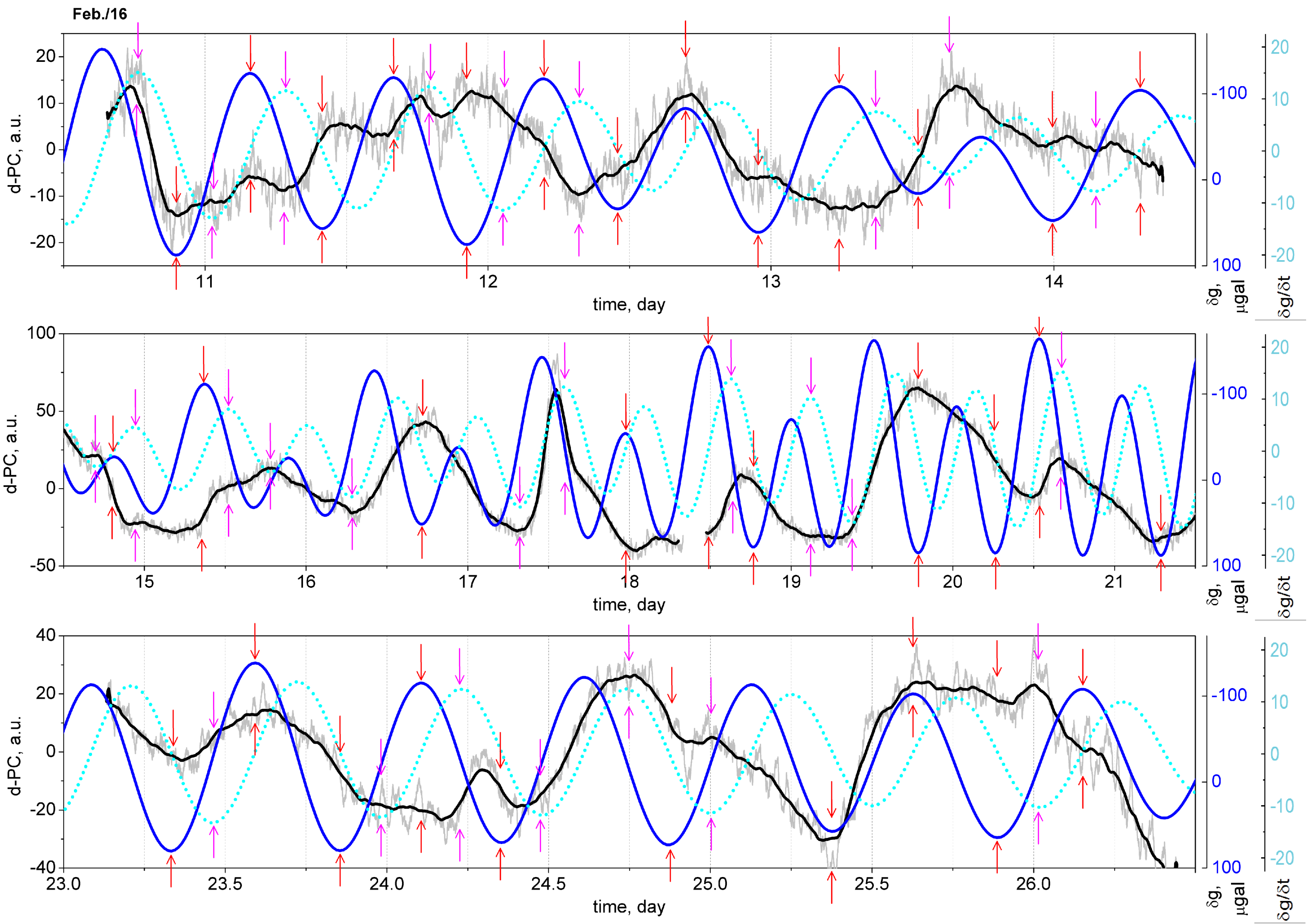
Figure 1 – Detrended photon-count (d-PC, with local smooth of 10^2 (grey line) and 10^3 points (black line)) and local gravimetric tide (δg, blue line) and first derivative ($\delta g/\delta t$, point, light-blue line) data for germination tests of coffee beans in optimal conditions, Feb./16. Pairs of vertical arrows (same time) mark significant turning points of δg (red arrows) or to $\delta g/\delta t$ (purple arrows) in coincidence to local/trend changes in d-PC data.

Figure 2 – Detrended photon-count (d-PC, with local smooth of 10^2 (grey line) and 10^3 points (black line)) and local gravimetric tide (δg, blue line) and first derivative ($\delta g/\delta t$, point, light-blue line) data for germination tests of coffee beans in optimal conditions, March 2016. Pairs of vertical arrows (same time) mark significant turning points of δg (red arrows) or to $\delta g/\delta t$ (purple arrows) in coincidence to local/trend changes in d-PC data. Test t3 (23d to 26d) have vertical axis (d-PC) divided by 3, to fit similar scale of other tests.

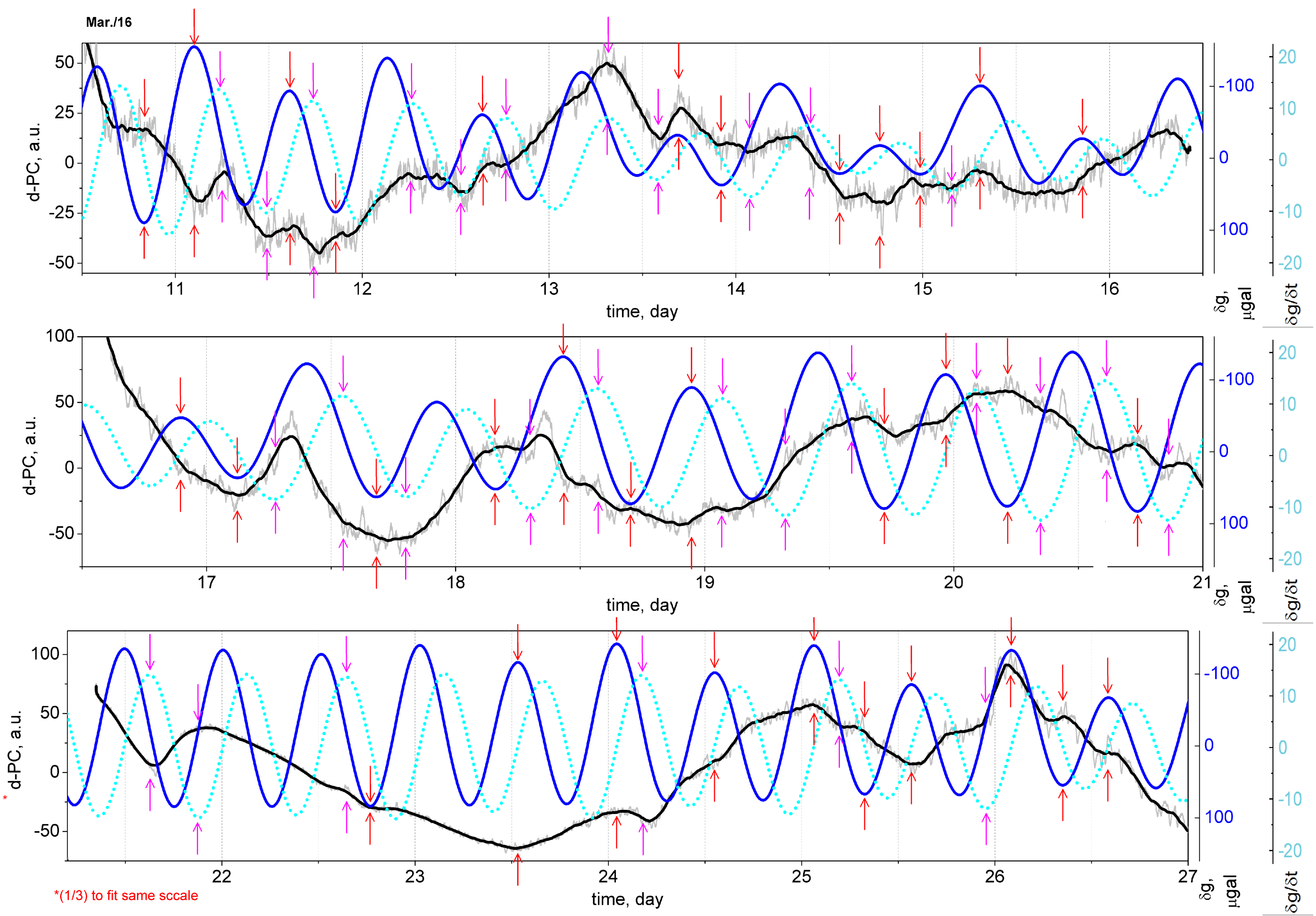
Figure 3 – Periodograms for the d-PC data (black curves) and gravimetric function δg (blue curves), with normalized amplitude, for the following tests: February - t1 (10.5d-14.5d), t2(14.5d-21.5d) and t3 (23d-27d); March – t1(10.5d-16.5d), t2(16.5d-21d) and t3 (21.5d-27d), in linear (upper panels) and logarithmic (lower panels) scales.

Figure 4 – Illustration for a model of microtubule/microfilament networks in the root cap, highlighting some examples of longitudinal lanes (blue), U-like lanes (red) and lateral, transverse lanes (orange), with respective arrow axis of maximum response (by colour). Draw over original micrograph of Barlow (2003): root-cap boundary (black arrows), protoderm initials (a), columella initials (c), quiescent centre (q), statenchyma (S) with prominent starch grains and forming part of the group of axially oriented cells which comprise the columella, lateral root cap (L); the star (*) indicates a possible site from which

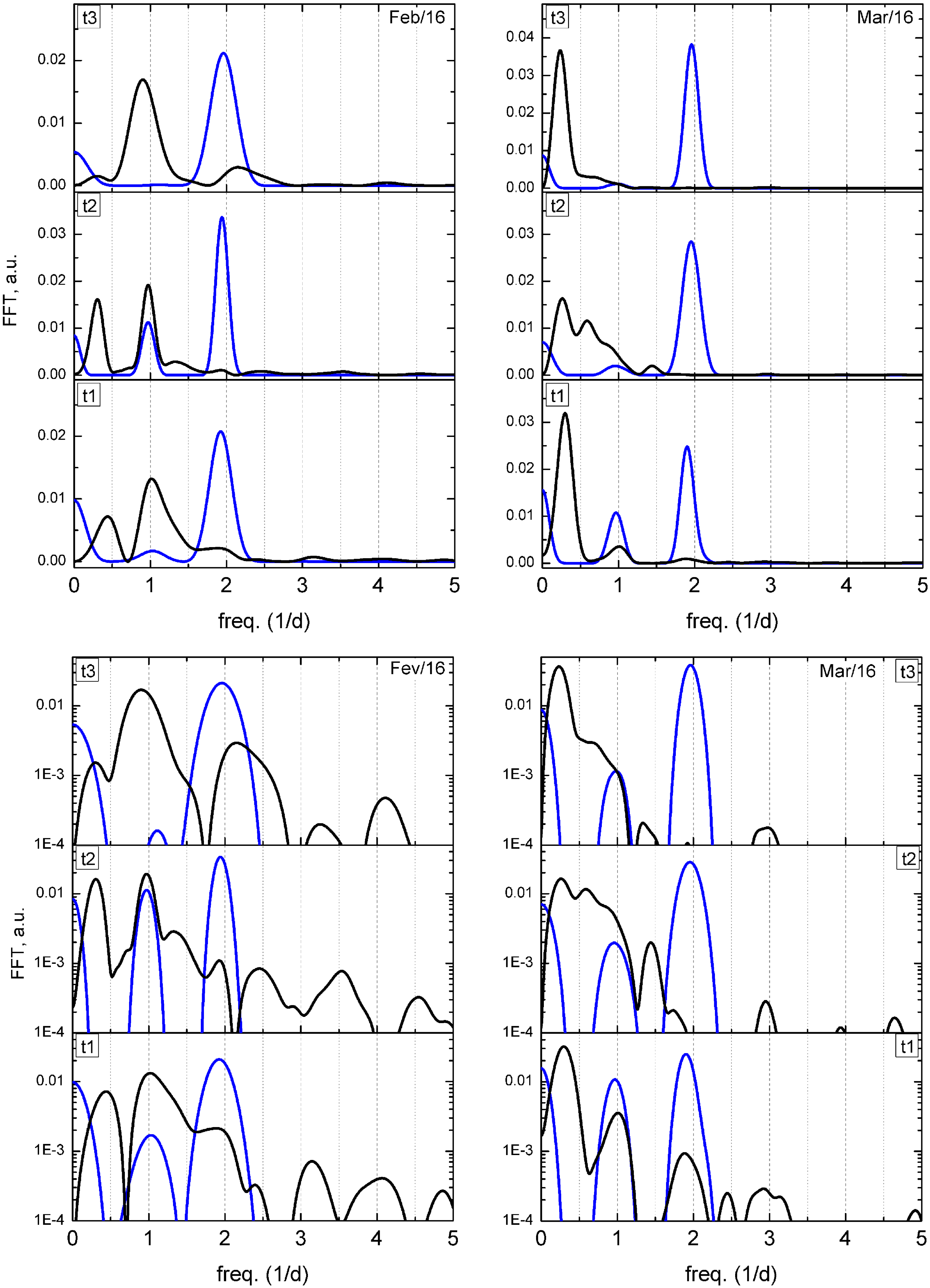
a cell has detached.



Feb./16
d-PC, a.u.
time, day
δg, μgal
δg/δt



Mar./16
d-PC, a.u.
time, day
δg, μgal
δg/δt
* d-PC, a.u.
*(1/3) to fit same sccale



t3
Feb/16
t2
t1
FFT, a.u.
freq. (1/d)
t3
Mar/16
t2
t1
freq. (1/d)
t3
Fev/16
t2
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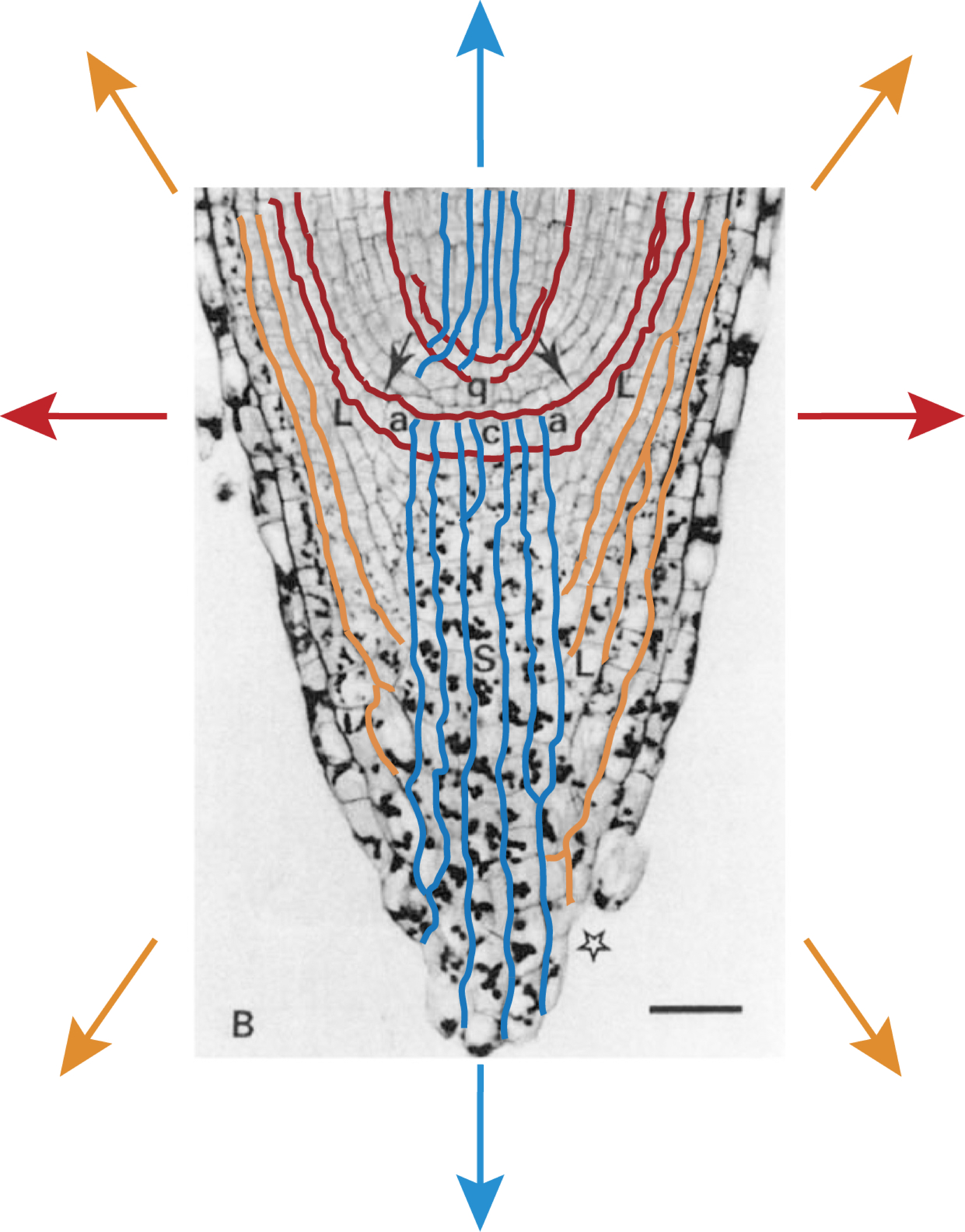
FFT, a.u.
freq. (1/d)
Mar/16
t3
t2
t1
freq. (1/d)



q
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