

THE GENETICS AND EVOLUTION OF SOLAR TRACKING

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ABSTRACT

The heliotropic movement of sunflower shoots, also known as solar tracking, is a dramatic example of a diurnal rhythm in plant growth. The shoot apex continuously tracks the sun's position in the sky as it changes from east at dawn to west at dusk over the course of the day. At night, the apex reorients back to an eastward orientation. As a sunflower reaches reproductive maturity, these cycles dampen, and disks predominantly maintain an eastward orientation at anthesis. Though these phenomena have long been observed, the developmental and molecular mechanisms by which external cues and internal rhythms are integrated to produce these diurnal patterns of growth are largely unknown. We have taken developmental and natural variation approaches at multiple evolutionary scales to understand the physiology, genetics, and diversity of these traits. Manipulative studies implicate the circadian clock as a driver of nocturnal reorientation and as a regulator of mature head orientation. Through phenotyping an association mapping panel of 280 cultivated sunflower lines with time-lapse imaging in the field, we have described ample diversity in the mean and variance of the diurnal phase of solar tracking movements and the orientation of mature disks, and we have identified several SNPs significantly associated with multiple solar tracking parameters. Finally, a survey of other diploid *Helianthus* species reveals that solar tracking is common among annuals and perennials with broad distributions but not found in basal rosette perennials of the southeastern US, suggesting this trait likely evolves as a component of a resource-acquisitive ecophysiological syndrome.

Key words: heliotropism, movement, circadian clock, phototropism, natural variation, association mapping

INTRODUCTION

Plants experience daily predictable cycles in the availability of resources and in the occurrence of environmental stresses. To cope with these oscillating environmental conditions, many aspects of plant growth, development, and physiology are adapted occur with diurnal rhythms such that peak activity coincides with the most favorable portion of a 24-h period. Although fluctuations of external cues like light or temperature may be the sole drivers of these diurnal plant traits, more often internal rhythms driven by the endogenous circadian clock also play an essential role in jointly coordinating these biological cycles (Alabadi and Blazquez, 2009; Harmer, 2009). Clock regulation is especially important for activities that must anticipate the availability of resources or the onset of environmental pressures, as waiting to directly experience these factors as cues may leave plants with insufficient time to mount fully effective responses, (e.g., activating metabolic or physiological defenses against diurnally active herbivores and pathogens; Wang et al., 2011).

Solar tracking, or heliotropism, of the growing stems of the common sunflower, *Helianthus annuus*, is perhaps the most conspicuous example of a diurnal growth trait in the plant kingdom (Vandenbrink et al., 2014; Kutschera and Briggs, 2016). During the day, the stem grows such that the shoot apex continuously reorients to remain normal to incident sunlight throughout the day, thus tracing a path from facing east at dawn to facing west at dusk (Fig. 1). The stem also reorients at night such that the shoot apex once again faces east in anticipation of dawn (Fig 1). Both movements appears to be largely driven by growth through irreversible cell expansion, as sunflower lacks specialized motor organs known as pulvini that promote reversible, turgor-driven heliotropism of leaves in other systems (Koller, 2001).

Heliotropic movement begins soon after sunflower seedlings begin expanding their true leaves but then slows as plants approach anthesis, at which point the plants stop tracking and maintain an easterly orientation until senescence (Shibaoka and Yamaki, 1959; Lang and Begg, 1979). This final point has been subject to a long-running misconception. For centuries, many authors have erroneously stated that mature heads do track the sun (e.g., Gerarde, 1597; Kircher, 1667; Koller, 2011), leading those who have then failed to observe floral heliotropism to dismiss the phenomenon entirely (Gerarde, 1597; Meehan, 1884; Kellerman, 1889). However, seminal studies corrected the literature by publishing photographic evidence of the daily movements of young plants (Schaffner, 1898, 1900).

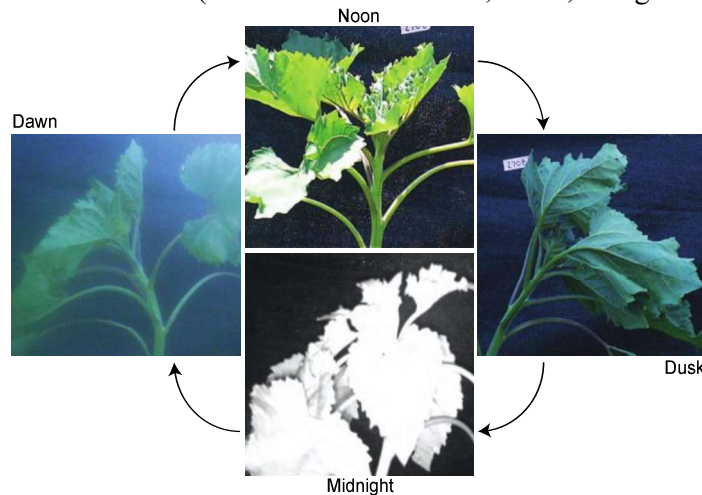


Fig. 4: Solar tracking and nocturnal reorientation of the sunflower stem. East was to the left and west to the right of the plant filmed in this series. Midnight photo taken with infrared LED flash built into camera.

Although the solar tracking of sunflower stems has been observed for centuries, the biological mechanisms that govern this behavior and the evolutionary history of the trait have received little attention (Shibaoka and Yamaki, 1959; Vandenbrink et al., 2014; Kutschera and Briggs, 2016). For instance, while we know a moving light source is a critical driver of heliotropic bending, how this signal from the changing relative position of the sun is perceived and how it leads to differential growth of lateral stem segments paced to the sun's east-to-west trajectory are largely unknown. Moreover, although an endogenous rhythm has been implicated in the regulation of solar tracking because plants rotated 180° take several days to fully match their growth to the new orientation (Shibaoka and Yamaki, 1959), the nature of this rhythm and its interactions with environmental signals are also not understood. Finally, the evolutionary history of solar tracking, the abundance of natural variation in this trait, as well as the ecological functions of heliotropism and the eastward orientation of mature disks have been little explored.

Here, we discuss what is known with respect to the first two physiological questions and also report several aspects of our work in progress that aims to address the final evolutionary question using a variety of approaches. First, we review previous studies on the regulation of solar tracking. Then, we report an initial assessment of natural variation in the timing of nocturnal reorientation using a recently generated association mapping panel of cultivated

sunflower. Finally, we discuss how our preliminary survey of diversity in solar tracking within the genus indicates how solar tracking may serve as part of a larger ecophysiological syndrome adapted for resource acquisition.

POSSIBLE MECHANISMS REGULATING SOLAR TRACKING

Surprisingly little has been published on the physiological mechanisms underlying solar tracking despite the long period over which this trait has been recognized (Schaffner, 1898, 1900; Vandenbrink et al., 2014; Kutschera and Briggs, 2016). Given that sunflowers do not have pulvini, it is very likely that the movements of solar tracking stems are due to asymmetric growth on the two sides of the stem, as has been reported for the petioles of leaves undergoing rhythmic ‘sleep movements’ (Pfeffer, 1903). The coincident timing with which solar tracking and leaf cell expansion cease at anthesis has also led several authors to infer that solar tracking is a growth mediated process (Lang and Begg, 1979; Koller, 2001). However, unlike rhythmic leaf movements, the initiation of solar tracking requires cues from the environment. Strongly directional light is clearly required to drive stem movements during the day. Plants grown under stationary overhead light in greenhouses or growth chambers do not track (Shell and Lang, 1976; B. Blackman, S. Harmer, personal observation), and several investigators have reported instances in which young plants have failed to track on cloudy or rainy days (Schaffner, 1898; Shibaoka and Yamaki, 1959). It is very likely that the daily east-to-west movements of sunflower plants is auxin-mediated and is initiated by the well-studied phototropin signaling pathway (Fankhauser and Christie, 2015).

However, no strong directional light source exists in nature that can explain the stereotyped west-to-east nocturnal reorientation of sunflower stems. We suggest that this directional movement at night in anticipation of dawn may be generated by circadian regulation of growth pathways. Several lines of evidence support this possibility. For instance, resetting of solar tracking movements takes several days when plants are experimentally rotated 180° during the night (Shibaoka and Yamaki, 1959). In addition, under long day photoperiods, the speed of stem movement must be and is substantially more rapid at night than during the day for the shoot apex to face east by dawn (Schaffner, 1898, 1900; Vandenbrink et al., 2014; Kutschera and Briggs, 2016). Finally, in some instances developing buds have been observed to achieve their eastward orientation well ahead of dawn (Shell and Lang, 1975; B. Blackman, personal observation). These observations all suggest involvement of an endogenous mechanism in solar tracking.

We therefore predict that the circadian clock provides the mechanistic basis for the endogenous rhythms that interact with directional light signaling and other environmental cues to drive solar tracking and nocturnal reorientation. In particular, we expect that the circadian clock drives diurnal rhythms in the abundance or activity of light signaling components and hormones that drive differential stem growth (Foster and Morgan, 1995; Millar and Kay, 1996; Jouve et al., 1999; Covington et al., 2008). The circadian clock may also gate how responsive plants are to these stimuli at particular times of day, following a paradigm that has been developed through the study of plant growth and organ expansion in controlled environmental conditions (Covington and Harmer, 2007; Nozue et al., 2007; Arana et al., 2011). We are currently conducting organismal and molecular experiments that will allow us to better understand the physiological mechanisms underlying solar tracking under naturally fluctuating field conditions and, in doing so, to determine whether the circadian clock does in fact play an instrumental role in governing one or more aspects of this fascinating plant growth behavior.

ASSOCIATION MAPPING IDENTIFIES NATURAL VARIANTS ASSOCIATED WITH SOLAR TRACKING

Natural variation can also provide a useful entry point to begin connecting genotype to phenotype and thus to understand the molecular basis of particular traits. We have complemented our ongoing developmental studies by taking an association mapping approach to further characterize the molecular mechanisms that regulate solar tracking. Concerted efforts by the Compositae Genome Project and the Sunflower Genome Consortium over the past decade have produced a panel of 288 lines that harbor ~90% of the common alleles segregating in cultivated sunflower (Kane et al., 2011; Mandel et al., 2011, 2013; Bachlava et al., 2012; Bowers et al., 2012). This panel is a tremendous resource. Because the genotypes are known and the lines are largely inbred and homozygous, any phenotype that can be scored on the panel can be quickly associated with single nucleotide polymorphisms (SNPs). Moreover, because this panel has been thoroughly genotyped by a succession of genomic methods over time with release of whole-genome resequencing data for the whole panel imminent, the genotypic resolution for association mapping is becoming comprehensive and high-resolution (Mandel et al., 2013; Nambeesan et al., 2015).

We have phenotyped the sunflower association mapping panel for solar tracking at a field plot at Morven Farm, VA, a property owned by the University of Virginia Foundation. Because filming all lines concurrently was prohibitively costly and difficult, we planted three replicates per line across a series of fifteen staged plants. Replicates were evenly distributed such that each accession had one replicate grown in the first third of the plantings, one in the middle third of the plantings, and one in the final third of the plantings. For a given replicate, three seeds were sown in a five-gallon paint bucket containing local soil mixed with 10% compost and with several holes drilled in the bottom for drainage. Plants were watered once or twice daily dependent on local conditions and plant size, and thinning was performed two weeks after germination.

Plants were filmed ~5 weeks on average after sowing, during the developmental period after budding but well before anthesis for most accessions. For filming, the buckets were placed in front of a matte black backdrop, and we used Bushnell X-8 trail cameras to capture images every 10 min for 48 to 72 h. The resulting time-lapse videos were visually evaluated for several traits, including the timing of nocturnal reorientation (i.e., the time relative to dusk when the stem first appears to move eastward instead of westward). The compass orientation of heads at anthesis was also scored on all plants. Association mapping was conducted for the means and coefficients of variation for each trait using a mixed-linear model that controlled for population structure and kinship in TASSEL v3.0 (Bradbury et al., 2007; Zhang et al., 2010). Genotypic data for the panel consisted of ~5.8K SNPs previously scored using an Illumina Infinium SNP array (Mandel et al., 2013).

We observed abundant variability in the timing of nocturnal reorientation in the association mapping panel. While the majority of lines began nocturnal reorientation within 30 minutes before or after dusk (mean = -6.2 ± 2.5 min), a notable number of lines began nocturnal reorientation over an hour earlier or later than dusk (Fig. 2A). The variability of this trait within lines also varied among lines. That is, for lines where three replicates were scored, we observed that the standard deviation in the timing of nocturnal reorientation ranged from 2 min to 2 h.

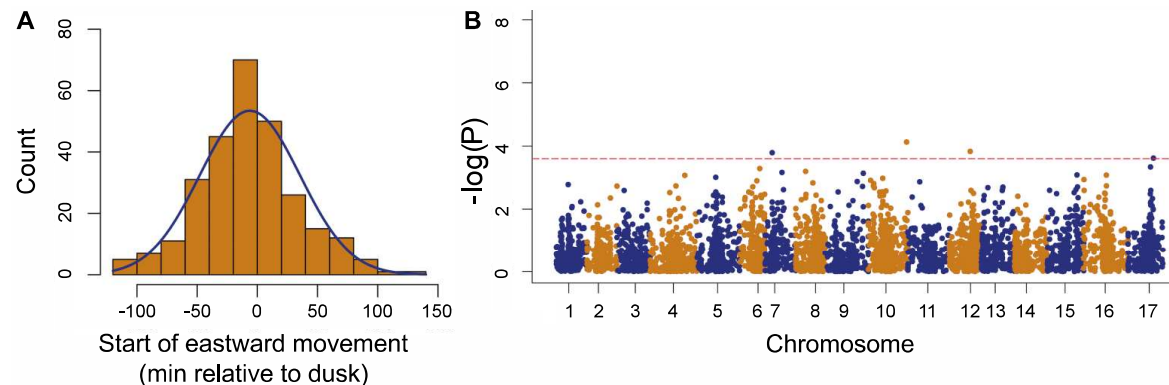


Figure 5: Phenotypic and genetic variation in timing of nocturnal reorientation. (A) Distribution of 288 cultivated sunflower lines scored by time-lapse photography for the time relative to dusk when the stem begins to reorient toward East. (B) Manhattan plot illustrating the significance level of associations tests for ~5.8K SNPs with the timing of nocturnal reorientation. Dashed red line indicates significance threshold after correction for multiple tests.

Association mapping yielded several SNPs significantly associated with variation in the mean timing of nocturnal reorientation (Fig. 2B). The significantly associated SNPs are located in annotated transcripts homologous to a mitochondrial ATP synthase G subunit family protein, NAD(P)H-quinon oxidoreductase subunit L, a DnaJ domain transcription factor, and a DTW domain-containing protein. We also detected several SNPs associated with variability in mature head orientation, including a homolog of the core circadian clock component *LATE ELONGATED HYPOCOTYL (LHY)*, possibly corroborating a role for the clock in solar tracking traits.

The limited number of significant SNPs observed may reflect the genetic architecture of intraspecific variation in this trait. Traits largely governed by many rare alleles and/or common alleles of moderate effect typically show similar patterns. However, these findings may also reflect the limited sampling of genomic space provided by the current genotypic dataset. We expect the strength of our approach to improve as the full resequencing dataset for the association mapping panel becomes available. That data will be very helpful for determining whether these genes or closely linked genes are best associated with the trait and thus most likely to have a causal influence. Moreover, we expect a sizable portion of the genome is not in strong linkage disequilibrium with any of the SNPs in the current sample, and thus there may be ample potential to detect additional significantly associated polymorphisms.

SOLAR TRACKING: A RESOURCE-ACQUISITIVE ECOPHYSIOLOGICAL SYNDROME TRAIT?

Solar tracking has been most remarked upon and studied in wild and cultivated populations of the common sunflower, *Helianthus annuus*. However, Schaffner also observed

solar tracking of the stems of two other wild *Helianthus* species over 100 years ago (Schaffner, 1898, 1900). These old observations raise several questions. How far back in the sunflower lineage did this behavior evolve? Is solar tracking evolutionarily labile? Does solar tracking demonstrate correlated evolution with other characters as part of a broader ecophysiological syndrome?

To address these questions, we filmed a subset of the diploid *Helianthus* species during the summers of 2014 and 2015 at our field site at Morven Farm outside of Charlottesville, VA, USA. Seeds were scarified and germinated on moist Whatman paper in Petri dishes in the dark for up to 7 days. After one day of light exposure, the seedlings were transplanted into cell packs containing a 1:1 mixture by weight of Fafard 3B soil and calcined clay. Seedlings were raised for up to four weeks in the University of Virginia Greenhouses under 16 h days before transplantation into the ground or into buckets filled with soil at our field site. Stems were filmed for 72 to 96 h during the developmental period after budding but before anthesis. Images captured every 5 or 10 min, and the resulting time-lapse videos were visually evaluated for evidence of tracking.

A revised, generally well resolved phylogeny of diploid *Helianthus* developed through sequencing and analysis of 170 nuclear genes was recently published (Fig. 3; Stephens et al., 2015). When considered on this tree, our preliminary findings show a striking pattern of character evolution for solar tracking. The phylogeny resolves the genus into three major clades: annuals, erect perennials with widespread distributions in North America, and perennials mostly endemic to the southeastern United States that often grow as basal rosettes. In our diversity survey, we observed solar tracking for all members sampled from both the annual and widespread perennial clades (Fig. 3). We also observed solar tracking for another member of the widespread perennial clade not included in the diploid tree because the species consists of both diploid and polyploid populations, *H. decapetalus*, and Schaffner reported tracking of the polyploid *H. pauciflorus*, which belongs to this clade as well (Schaffner, 1898). In contrast, we did not observe solar tracking for any of the members of the southeastern perennials sampled or for additional closely related but poorly resolved perennial species (Fig. 3). Although some of these taxa do grow as basal rosettes (*H. atrorubens*, *H. radula*, *H. occidentalis*), others do not (*H. floridanus*, *H. mollis*). Thus the pattern we observe cannot be explained solely by constraints on internode elongation during the period of active leaf expansion.

Notably, a recent macroevolutionary analysis reported similar phylogenetic patterns for many leaf economics spectrum and resource use traits (Mason and Donovan, 2015). That is, correlated patterns of evolution were observed such that the annual and widespread perennial clades appear to evolve a correlated syndrome of resource-acquisitive trait values (e.g., deltoid leaves, greater vein length per unit area, higher stomatal conductance). In contrast, the southeastern perennial clade appears to evolve toward a syndrome of resource-conservative trait values (i.e., lanceolate or acuminate leaves, lower vein length per unit area, lower stomatal conductance). If more comprehensive sampling confirms the similar preliminary pattern we observe for solar tracking, then these findings would corroborate the hypothesis that solar tracking serves a critical function in enhancing resource acquisition, a longstanding idea that has been difficult to test empirically. Because we have not been able to grow and film an outgroup to the genus and yet observe tracking of *H. porteri*, the most basally diverging taxon within the genus, the important question of when and in what lineage solar tracking first evolved remains unresolved. In addition, due to poor resolution of branching events ancestral to the southeastern perennial clade, some uncertainty remains about how

strictly congruent the transition to a resource-conservative ecophysiological syndrome is with the evolutionary loss of solar tracking.

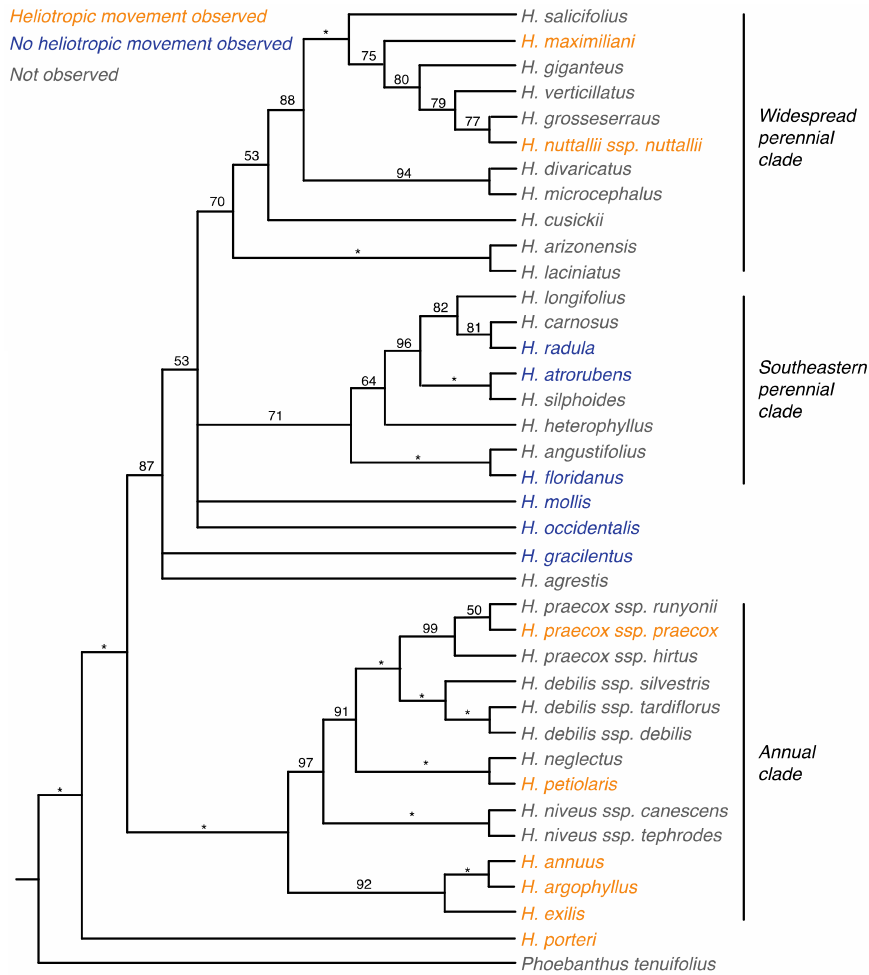


Figure 6: Phylogenetic survey of solar tracking. Species names are colored by trait status (see inset). Figure adapted from Stephens *et al.* 2015. We also observed that a diploid accession of *H. decapetalus*, a member of the widespread perennial clade not included in the species tree does exhibit solar tracking. The species tree was constructed with Maximum Pseudo-likelihood Estimation of the Species Tree v1.4 (MP-EST; Liu *et al.* 2010). Bootstrap support provided for nodes, asterisks indicate bootstrap support = 100. Nodes with <50 bootstrap support collapsed.

CONCLUSIONS AND FUTURE DIRECTIONS

It has been extensively shown in several systems under controlled conditions that the interaction of the circadian clock with external signals drives diurnal cycles of light signaling components and hormones that play essential roles in directional plant growth. By focusing on solar tracking as a model system, we are working to determine whether this paradigm also holds true for a growth trait that impacts plant fitness in changing natural environments. Natural variation shows great promise as an experimental means of learning about these underlying mechanisms, and we expect the release of whole genome resequencing data for the cultivated sunflower association mapping panel to enhance these efforts dramatically. In

addition, the diversity in solar tracking that we have observed among *Helianthus* species appears to provide insight into the function of solar tracking as part of an ecophysiological syndrome of evolutionary correlated traits that enhance resource acquisition. Comparative developmental and transcriptomic studies across species that do and do not track may also prove a fruitful means of gaining understanding into the mechanisms that regulate this fascinating plant growth trait.

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LITERATURE

- Alabadi, D., and M.A. Blazquez. 2009. Molecular interactions between light and hormone signaling to control plant growth. *Plant Mol Biol* 69: 409–17.
- Arana, M.V., N. Marin-de la Rosa, J.N. Maloof, M.A. Blazquez, and D. Alabadi. 2011. Circadian oscillation of gibberellin signaling in Arabidopsis. *Proc Natl Acad Sci U S A* 108: 9292–7.
- Bachlava, E., C.A. Taylor, S. Tang, J.E. Bowers, J.R. Mandel, J.M. Burke, and S.J. Knapp. 2012. SNP Discovery and Development of a High-Density Genotyping Array for Sunflower. *PLoS ONE* 7: e29814.
- Bowers, J.E., E. Bachlava, R.L. Brunick, L.H. Rieseberg, S.J. Knapp, and J.M. Burke. 2012. Development of a 10,000 Locus Genetic Map of the Sunflower Genome Based on Multiple Crosses. *G3* 2: 721–729.
- Bradbury, P.J., Z. Zhang, D.E. Kroon, T.M. Casstevens, Y. Ramdoss, and E.S. Buckler. 2007. TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics* 23: 2633–2635.
- Covington, M.F., and S.L. Harmer. 2007. The circadian clock regulates auxin signaling and responses in Arabidopsis. *PLoS Biol* 5: e222.
- Covington, M.F., J.N. Maloof, M. Straume, S.A. Kay, and S.L. Harmer. 2008. Global transcriptome analysis reveals circadian regulation of key pathways in plant growth and development. *Genome Biol* 9: R130.
- Fankhauser, C., and J.M. Christie. 2015. Plant phototropic growth. *Curr Biol* R384–389.
- Foster, K.R., and P.W. Morgan. 1995. Genetic Regulation of Development in Sorghum bicolor (IX. The ma3R Allele Disrupts Diurnal Control of Gibberellin Biosynthesis). *Plant Physiol* 108: 337–343.
- Gerarde, J. 1597. The Herball or Generall Historie of Plantes. John Norton, London.
- Harmer, S.L. 2009. The circadian system in higher plants. *Annu Rev Plant Biol* 60: 357–77.

- Jouve, L., T. Gaspar, C. Kevers, H. Greppin, and R. Degli Agosti. 1999. Involvement of indole-3-acetic acid in the circadian growth of the first internode of *Arabidopsis*. *Planta* 209: 136–42.
- Kane, N.C., N. Gill, M.G. King, J.E. Bowers, H. Berges, J. Gouzy, E. Bachlava, et al. 2011. Progress towards a reference genome for sunflower. *Botany* 89: 429–437.
- Kellerman, W.A. 1889. Observations on the Nutation of Sunflowers. *Transactions of the Annual Meetings of the Kansas Academy of Science* 12: 140–158.
- Kircher, A. 1602-1680. 1667. Athanasij Kircheri e Soc. Iesu. Magneticum naturae regnum siue Disceptatio physiologica de triplici in natura rerum magnete, ... Ad inclytum, & eximium virum Alexandrum Fabianum . [Rome]: [publisher not identified], [1667]. Available at: <https://search.library.wisc.edu/catalog/9911147779402121>.
- Koller, D. 2001. Solar navigation in plants. In D.-P. Häder, and M. Lebert [eds.], *Photomovement*, 833–896. Elsevier, Amsterdam, Netherlands.
- Koller, D. 2011. *The restless plant*. Harvard University Press, Cambridge, MA.
- Kutschera, U., and W.R. Briggs. 2016. Phototropic solar tracking in sunflower plants: an integrative perspective. *Ann Bot* 117: 1–8.
- Lang, A.R.G., and J.E. Begg. 1979. Movements of *Helianthus annuus* leaves and heads. *J Appl Ecol* 16: 299 – 305.
- Liu, L., L. Yu, and S.V. Edwards. 2010. A maximum pseudo-likelihood approach for estimating species trees under the coalescent model. *BMC Evol Biol* 10: 1–18.
- Mandel, J., J. Dechaine, L. Marek, and J. Burke. 2011. Genetic diversity and population structure in cultivated sunflower and a comparison to its wild progenitor, *Helianthus annuus* L. *Theor Appl Genet* 123: 693–704.
- Mandel, J.R., S. Nambeesan, J.E. Bowers, L.F. Marek, D. Ebert, L.H. Rieseberg, S.J. Knapp, and J.M. Burke. 2013. Association Mapping and the Genomic Consequences of Selection in Sunflower. *PLoS Genet* 9: e1003378.
- Mason, C.M., and L.A. Donovan. 2015. Evolution of the leaf economics spectrum in herbs: Evidence from environmental divergences in leaf physiology across *Helianthus* (Asteraceae). *Evolution* 69: 2705–2720.
- Meehan, T. 1884. General Notes. *Bot Gaz* 9: 48–51.
- Millar, A.J., and S.A. Kay. 1996. Integration of circadian and phototransduction pathways in the network controlling CAB gene transcription in *Arabidopsis*. *Proc Natl Acad Sci U S A* 93: 15491–6.
- Nambeesan, S.U., J.R. Mandel, J.E. Bowers, L.F. Marek, D. Ebert, J. Corbi, L.H. Rieseberg, et al. 2015. Association mapping in sunflower (*Helianthus annuus* L.) reveals independent control of apical vs. basal branching. *BMC Plant Biol* 15: 84.
- Nozue, K., M.F. Covington, P.D. Duek, S. Lorrain, C. Fankhauser, S.L. Harmer, and J.N. Maloof. 2007. Rhythmic growth explained by coincidence between internal and external cues. *Nature* 448: 358–61.
- Pfeffer, W. 1903. *Physiology of Plants*. Oxford University Press, Oxford.
- Schaffner, J.H. 1898. Observations on the nutation of *Helianthus annuus*. *Bot Gaz* 25: 395–403.

- Schaffner, J.H. 1900. The Nutation of Helianthus. *Bot Gaz* 29: 197–200.
- Shell, G.S.G., and A.R.G. Lang. 1975. Description of leaf orientation and heliotropic response of sunflower using directional statistics. *Agr Meteorol* 15: 33–48.
- Shell, G.S.G., and A.R.G. Lang. 1976. Movements of sunflower leaves over a 24-h period. *Agr Meteorol* 16: 161–170.
- Shibaoka, H., and T. Yamaki. 1959. Studies on the growth movement of sunflower plant. *Scientific Papers of the College of General Education of the University of Tokyo* 9: 195–126.
- Stephens, J.D., W.L. Rogers, C.M. Mason, L.A. Donovan, and R.L. Malmberg. 2015. Species tree estimation of diploid Helianthus (Asteraceae) using target enrichment. *Am J Bot* 102: 910–920.
- Vandenbrink, J.P., E.A. Brown, S.L. Harmer, and B.K. Blackman. 2014. Turning heads: the biology of solar tracking in sunflower. *Plant Sci* 224: 20–26.
- Wang, W., J.Y. Barnaby, Y. Tada, H. Li, M. Tor, D. Caldelari, D. Lee, et al. 2011. Timing of plant immune responses by a central circadian regulator. *Nature* 470: 110–114.
- Zhang, Z., E. Ersoz, C.-Q. Lai, R.J. Todhunter, H.K. Tiwari, M.A. Gore, P.J. Bradbury, et al. 2010. Mixed linear model approach adapted for genome-wide association studies. *Nat Genet* 42: 355–360.