New Phytologist

Commentary

Winter is coming and the clock starts ticking

The passage from the vegetative to the flowering stage is a crucial life-history trait in plants, and reproductive success depends to a large extent on getting the timing right, so flowers and seeds are produced when pollinators are present and the conditions are right for the development of flowers, fruits and seeds (Amasino & Michaels, 2010). Plants have to rely on cues from the environment to determine the right moment to initiate flowering, and daylength and temperature are the main cues used in temperate environments with distinct cold and warm seasons. Combining long-term observations on flowering phenology with meteorological data can give us insight in how overwintering plants react to year-to-year variation in weather patterns and to climate change (Inouye *et al.*, 2002). In this issue of *New Phytologist*, Jánosi *et al.* (2020; pp. 1535–1547) not only use an extensive dataset of up to 33 years of observations on the first flowering date of no less than 329 bulbous perennial taxa at a single location in Hungary, the private garden of the former director of the Botanical Garden of the Eötvös Loránd University in Budapest, but they also present an elegant solution for a major problem that has always hampered the analysis of this kind of data.

> *'The results suggest that a cold spell in November, generally accompanied by snow, starts a kind of countdown clock towards flowering by triggering the onset of vernalization.'*

Year-to-year variation in flowering time due to fluctuations in temperature is a common observation, and one century ago, Francis Darwin published several 'Studies on phænology' in which he tried to link these observations to the weekly mean temperature and its deviations from normal (Darwin, 1919, 1921, 1922). As Darwin noted ('My phænological work was begun in 1917, so that I have only the record of four years with which to compare the facts of 1921'; Darwin, 1922, p. 34), the amount of phenological data obtained even in very long-running series of observations is limited: each year only gives a single data point for the start of flowering of a given species or population. Temperature, however, varies continuously, and not a single year is exactly the same: Francis Darwin presented up to 52 data points per year in his papers, a weekly average temperature for each week. How can you determine which day or week of the year really matters, when the number of variables (temperature per day or week) is much higher than the number of flowering dates (one per year)? Calculating individual correlations between each variable and flowering date (one X, one Y) leads to spuriously significant results: with an alpha of 0.05, one in every 20 tests can be significant purely by chance (Type I error), so even using weekly averages will give two or three false positives. Multivariate techniques (many X, one Y) can only be used when the number of data points (years) is higher than the number of variables.

A possible solution is to summarize the data and reduce the number of variables: daily or weekly data are compiled into monthly averages. Unfortunately, this leads to a decrease in sensitivity: a month with two very hot and two very cold weeks has normal monthly average temperature, the extremes are levelled out. The innovative solution used by Jánosi *et al.* is machine learning: the computer is asked to find an optimal estimator explaining year-by-year fluctuations in flowering date for each species individually, and they did this with monthly means, daily values and deviations from normal for 16 variables including precipitation, temperature at different heights and depths, snow cover and soil humidity.

Phenological studies that are based on multiple taxa often include a variety of growth forms (e.g. Fitter *et al.*, 1995), but Jánosi *et al.* focus on a specific life form, namely bulbous perennials. These plants, also called geophytes, are plants that often flower early in spring and only have aerial parts for a few months of the year. During that time, photosynthesis provides resources that are transported to the storage organ underground. Once the reserves are big enough, a flowering meristem forms and a complete inflorescence is formed during summer and autumn (Khodorova & Boitel-Conti, 2013). Exposure for several weeks to low temperatures, vernalization, is then needed before stalk elongation and flower development can take place (Wang *et al.*, 2019). This ensures that flowering only will take place after a prolonged cold period, but it gives no clue to the plant as to when winter is really over and flowering can start. As the inflorescence is already preformed, flowering does not require extensive cell division, only transport of water to the cells to make them expand (Veselý *et al.*, 2012). The taxa used in this study ranged in average flowering time from February to October.

The results of the study are quite surprising. First of all, monthly means were less good at predicting flowering time, probably because of the levelling effect, and this was to be expected. But the unexpected best predictor by far for flowering time was the daily snow depth anomaly, the deviation of the amount of snow present compared to the average value for that day of the year. For the early-flowering taxa (March–June), the best predictor was the snow depth anomaly on a spring day, which had a negative effect: more snow correlated to later flowering, but the snow depth anomaly on a day at the start of winter (early November) was a close second, with

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a positive effect (more snow correlated to earlier flowering). For the later flowering species, the influence of deviations in autumn snow cover was stronger than that of more or less snow in spring. It is important to realize that the days were numbered relative to the flowering date: in the worked-out example on *Ornithogalum lanceolatum*, the autumn day on which more snow than normal accelerated flowering the strongest was 166 days (5.5 months) before flowering, and the spring day that had the most effect (more snow leading to later flowering) was 35 days before the flowering date (19 April on average).

Of course, geophytes cannot sense the presence or absence of snow, especially since they have no aboveground parts during winter, but the deviation in snow depth appears to be an excellent proxy for the general weather situation in this Hungarian garden, not only on that particular day, but also on the days around it. The results suggest that a cold spell in November, generally accompanied by snow, starts a kind of countdown clock towards flowering by triggering the onset of vernalization. When the vernalization process is complete after two to three months, a lack of snow signals the end of winter and spring-flowering geophytes will start development towards flowering. The surprising fact that even in autumn-flowering geophytes, flowering time is accurately predicted by winter conditions almost a year earlier, suggests that they too mainly use this internal clock, which seems to be influenced only to a lesser extent by weather conditions in summer.

These results open the way to a whole suite of further research. First of all, this machine-learning method can be applied to already existing long-term datasets to see if similar results can be found in other geographical locations with different weather conditions. It is less likely to yield the same kind of results in regions where snowfall is relatively rare and average snow depth is close to zero, for instance close to the Atlantic coast of Western Europe, but other temperature-related variables might then become more apparent. Second, for a given species, gene expression could be studied in the autumn and spring periods that are pinpointed by this analysis, in tandem with precise local measurements of the variables that matter. This would finally allow us to extrapolate from the artificial, constant temperature environments that are often used in studies on vernalization (e.g. Wang *et al.*, 2019) to real-life conditions, with the added benefit of increasing our understanding of what climate change does to the timing of flowering.

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