A CORRELATION AND REGRESSION APPROACH TO PHENOLOGY

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ABSTRACT

In this paper the developmental stages leading up to flowering are reviewed. It is then shown how correlation and regression methods of analysis can be used to identify the influence of air temperature at different developmental stages, using snowdrop and clover as case studies. Hypotheses are proposed to account for the findings, and it is suggested how such hypotheses could be tested. The implications for the construction of a functional phenological classification of plants are discussed.

INTRODUCTION

Daily phenological observations in the form of first-flowering dates (FFDs) were recorded at the Royal Botanic Garden Edinburgh (RBGE) from 1850 to 1895, and have now been continued since 2002. In the current phase of the project, data are available over eight years for some taxa. This paper describes a provisional attempt to use correlation and regression methods of analysis to make sense of the observations.

As described in an earlier paper in this series (Harper and Morris, 2007) one aim of the project is to draw up a functional phenological classification for the purpose of predicting how different groups of plants are likely to react to any kind of climate change. To be useful as predictors, these groups need to be characterized on the basis of physiological properties. This means that it is not sufficient to measure phenological behaviour in current conditions and merely group plants together by similarity of flowering patterns, since the flowering of taxa – for instance various species of spring-flowering bulb – may be triggered by different internal and external conditions. In this case, they could react differently to any given change in climate that affects those conditions.

So the task facing us is not straightforward. Even if there were abundant data on flowering times and other phenological variables, knowledge of the mechanisms – the 'hidden phenology' as described in Sibbaldia 2 (Harper *et al.*, 2004) – is not available for most species. We are not ready to propose a classification at this stage, but we believe that correlation and regression analysis has the potential to identify some of the main physiological determinants of flowering behaviour in each taxon. We suggest a list of characteristics which could form the basis of a useful functional classification.

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The methods described have so far been developed on very few species, and so the paper is built around just two case studies – Common snowdrop, *Galanthus nivalis* and White clover, *Trifolium repens*. These will illustrate the potentials – and the pitfalls – of the method.

DEVELOPMENT OF RERPODUCTIVE SHOOTS

It was argued in Sibbaldia 5 (Harper and Morris, 2007) that, in some plants at least, there is an extended 'run-up' to flowering, during which environmental conditions may exert an influence at more than one stage of development. In the case of woody perennials there may be ten or more such stages, if we include vegetative development of the stem on which floral initiation later takes place.

The first illustration in that paper summarized the patterns of correlations between FFD or leafing date and average temperatures of preceding months, as revealed in other studies. Many species show negative correlations of FFD or leafing date with temperatures in the months immediately preceding flowering or leafing, these months lying mostly in the period December–April. A negative correlation between two sets of numbers means that as one decreases the other increases; a positive correlation would indicate that as one increases the other also increases; complete correlation is shown by a correlation coefficient of +1.0 or –1.0, and zero means no association between the sets of numbers. The most obvious explanation for a negative correlation in this case is that higher temperatures speed up development, so causing flowers or buds to open earlier. This relationship between FFD or leafing date and temperatures will be referred to as 'thermal acceleration'.

A rather smaller number of taxa show positive correlations between FFD and monthly average temperatures 4–10 months before flowering, usually in August–November. It was suggested in Sibbaldia 5 that these positive correlations, meaning that higher temperatures are associated with later flowering, are the result of 'resource switching', involving resources within the plant being diverted from reproductive growth to vegetative growth in warmer conditions, thus delaying FFD. Another possibility concerns dormancy setting, since low temperature might disrupt this process, resulting in a reduced chilling requirement and consequently earlier flowering. It had earlier been suggested that "prolonged growth in autumn in some way interferes with the vernalization process for some early-flowering species" (Fitter *et al.*, 1995). The positive correlations for FFD, ignoring the results for budburst, fall mostly in August and October–November, and we now suggest that these may represent more than one physiological phenomenon. It is probably not realistic to expect a single explanation for these effects in summer and autumn, and we return to them below.

Earlier still, there are negative correlations between FFD and monthly average temperatures, usually in April–June of the year preceding flowering, and it was suggested in Sibbaldia 5 that these could represent the effects of temperature on the timing of floral initiation. Floral initiation is when a vegetative meristem becomes a reproductive meristem, from which flowers eventually develop. In some ways it makes sense to consider the reproductive structures as parasites growing on the vegetative plant since, once floral initiation has occurred, the reproductive structures compete with the vegetative parts of the plant for resources and may have very different responses to the environment.

These results suggest that the influence of weather on flowering behaviour should be considered up to a year before FFD. However it is known from the literature that conditions much earlier may have to be taken into account. For instance *Arum maculatum* inflorescences need 18 months of development (Halevy, 1989). In Peach, *Prunus persica*, flowering occurs normally only after a sequence of cold, warm, cold and warm periods, corresponding to two winters with following warm seasons. This is because chilling is required for floral initiation (several months below 10°C), and also for flowering (800–1500 hours below 5°C) (Went, 1961). In some varieties of Olive, *Olea europaea*, winter chilling is required for floral initiation, and one year later chilling is again needed for successful flowering (Therios, 2009).

Fig. 1 has been designed to help visualize this long sequence of developmental stages in relation to the external environment. It represents a 26-month spiral timeline covering a little over two annual cycles. The various shades of grey suggest the varying day/night lengths through the seasons, with summer at the top (summer solstice = week 25) and winter at the bottom (winter solstice = week 51). The flattened shape

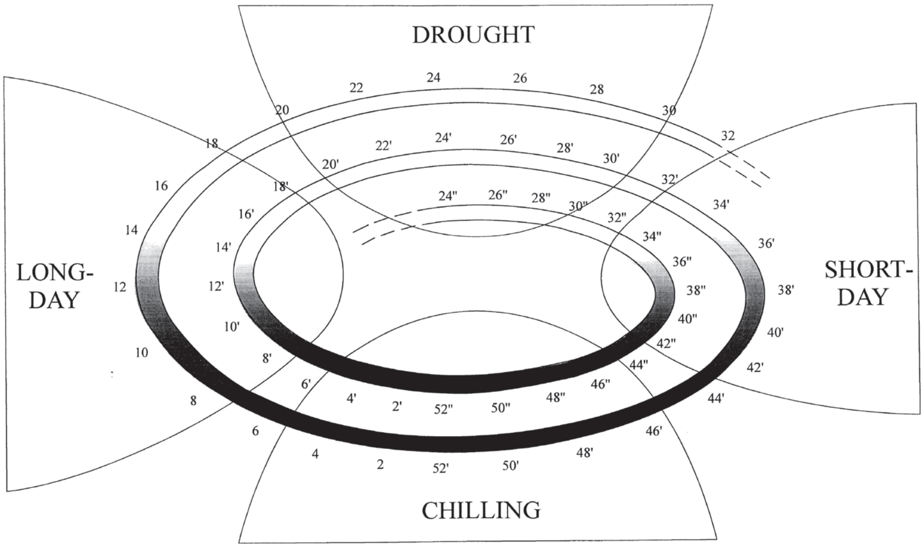

Fig. 1 The timeline spirals from the centre outwards. Shading suggests variation in day length through the seasons, while the tightness of the curve represents the rate at which average temperature and day length change. Weeks are numbered from January to December, plain numbers being used for the year of flowering and an apostrophe being added for each year earlier. The four outlined zones indicate when the respective environmental factors are most likely to affect the timing of flowering.

symbolically represents the fact that temperature and day and night length are changing rapidly around the spring and autumn equinoxes (weeks 12 and 38 respectively) in mid-March and mid-September, just as the direction of the timeline is rapidly changing at the left and right ends of the ellipse.

The diagram was originally prepared as a sequence for Powerpoint presentations. The timeline was displayed first on its own then successive slides were added showing four of the main environmental factors that might affect flowering behaviour. Thus, for plants grown in Edinburgh, chilling occurs in the autumn and winter months, and drought is most likely in summer. Long-day plants are most likely to respond to day length around the spring equinox, since that is when day length is changing most rapidly, and similarly for short-day plants in autumn. Day length changes slowly, if at all, in mid-winter and mid-summer, so that it is less likely that a plant will make use of changing day length in those seasons.

The next few sections of the paper discuss some of the main environmental influences determining the timing of flowering, using correlation and regression in *Galanthus nivalis* (Common snowdrop) and explaining the method as we go along. Since photoperiod is probably not used by snowdrops, the second case study is then brought in to illustrate possible photoperiod effects and at the same time further features of the method.

THERMAL ACCELERATION

The environmental factor to which most attention has been paid in other phenological studies is the thermal acceleration that occurs immediately before FFD in spring-flowering taxa. It is expected to have its effect for a few weeks or months right up to the commencement of flowering, and should appear as negative correlations between FFD (expressed as a Julian date, in other words counting days from 1 January) and average temperature for each month or other period. The basic approach in our method is therefore to calculate correlations for each of the months leading up to FFD, using the daily maximum and minimum temperatures as measured at the RBGE meteorological station in the Garden, and also the daily mean temperature, which is the arithmetic average of the measured temperatures.

Table 1 shows the analysis of FFDs for one accession of *G.nivalis* (1992.1202A in

Fig. 2 *Galanthus nivalis*. The top four flowers are fully open. The unopened bud is in the nodding position. Photo: Lynsey Wilson.

	Galanthus nivalis D31 2002-09					**Galanthus nivalis D31** 2002-09					**Galanthus nivalis D31** 2002-09			
	and 1892-95					and 1892-95					and 1892-95			
	FFDs					1st **residuals**, FFD/**JanCYmean**					2nd residuals, 1st resid/**JunCYmean**			
			R					**R**					**R**	
		max	**mean**	**min**			**max**	**mean**	**min**			**max**	**mean**	**min**
yr -2	Jul	-0.25	-0.27	-0.26	yr -2	Jul	0.15	0.16	0.16	yr -2	Jul	0.06	0.11	0.17
	Aug	0.25	0.13	-0.03		Aug	0.51	0.41	0.18		Aug	0.64	0.57	0.32
	Sep	-0.13	-0.26	-0.38		Sep	0.20	0.17	0.11		Sep	0.16	0.15	0.13
	Oct	-0.08	-0.13	-0.18		Oct	0.31	0.26	0.20		Oct	0.23	0.23	0.22
	Nov	-0.68	-0.67	-0.62		Nov	-0.19	-0.21	-0.22		Nov	0.09	0.08	0.07
	Dec	-0.06	-0.07	-0.08		Dec	0.42	0.41	0.36		Dec	0.50	0.51	0.46
PY	Jan	-0.46	-0.53	-0.59	PY	Jan	-0.12	-0.16	-0.20	PY	Jan	0.09	0.08	0.06
	Feb	-0.21	-0.24	-0.23		Feb	-0.15	-0.20	-0.23		Feb	-0.07	-0.12	-0.16
	Mar	-0.18	-0.32	-0.48		Mar	-0.32	-0.31	-0.26		Mar	-0.06	0.00	0.07
	Apr	-0.34	-0.33	-0.30		Apr	-0.17	-0.10	-0.02		Apr	0.00	0.10	0.19
	May	-0.69	-0.70	-0.65		May	-0.18	-0.27	-0.33		May	0.06	-0.04	-0.14
	Jun	-0.66	**-0.75**	-0.68		Jun	-0.56	**-0.59**	-0.46		Jun	-0.02	**0.00**	0.03
	Jul	-0.50	-0.45	-0.30		Jul	-0.06	-0.06	-0.05		Jul	0.39	0.37	0.26
	Aug	-0.66	-0.71	-0.55		Aug	-0.41	-0.36	-0.20		Aug	-0.01	-0.01	-0.01
	Sep	-0.60	-0.56	-0.47		Sep	-0.33	-0.13	0.10		Sep	0.14	0.34	0.53
	Oct	-0.37	-0.31	-0.25		Oct	0.25	0.35	0.41		Oct	0.61	0.66	**0.68**
	Nov	-0.16	-0.08	0.01		Nov	0.44	0.51	0.56		Nov	0.66	**0.68**	0.67
	Dec	-0.37	-0.31	-0.21		Dec	-0.04	-0.05	-0.05		Dec	0.41	0.39	0.34
CY	Jan	-0.79	**-0.83**	**-0.86**	CY	Jan	0.05	**0.00**	-0.06	CY	Jan	0.41	0.36	0.29
	Feb	-0.78	-0.79	-0.75		Feb	-0.19	-0.18	-0.16		Feb	0.08	0.06	0.04
	Mar	-0.47	-0.40	-0.25		Mar	-0.11	0.07	0.29		Mar	-0.09	0.17	0.48

Table 1 Correlations between first-flowering dates of *Galanthus nivalis* and monthly average temperatures (left), first residuals (centre), and second residuals (right).

bed D31), covering eight years, and data for the same species from another four years (1892–95) to make up a more adequate data set. Although daily observations were made from 1850 to 1895 in the Garden, meteorological measurements to modern standards are available only from the late 1890s, meaning that FFDs only from the last four years can be used for this study.

The table on the left shows the correlation coefficients between FFD and each monthly average temperature back to the July of the second year before flowering (CY = current year; PY = previous year; second year before flowering = yr-2). The boxed cells indicate the period including the FFDs being analysed. Blue is used to show strong negative correlations, while yellow is used similarly for positive correlations. Bold font draws attention to results of interest. A correlation of 1.00 would indicate complete correspondence between temperature and FFD, while 0.00 would mean no relationship at all.

It should be noted that the use of colour and bold font is not intended to indicate statistical significance. In this paper we are not claiming to present any statistically significant results from the use of correlation and regression analysis on the so far inadequate data sets at our disposal. Instead we aim, at this stage, merely to argue that our phenological observations are compatible with explanations that we present in the form of hypotheses. These hypotheses will need independent testing which is discussed below.

The left-hand table of correlations shows high negative values for average JanuaryCY mean and minimum temperatures, and for the average mean temperature in JunePY. This pattern of negative correlations is to be expected since the JanuaryCY result presumably reflects thermal acceleration and the June correlation has already been reported by an independent study, as illustrated in Fig. 1 in Sibbaldia 5 (Sparks & Carey, 1995).

At this point it needs to be emphasized that a good deal of caution is required in interpreting patterns in these tables of correlations, especially when there is a small or otherwise inadequate data set. There are four reasons for exercising caution. First, there may be chance correlations between the temperatures in one period and those in another period. For instance there is a weak correlation between JanuaryCY mean and JunePY mean temperatures (R, the correlation coefficient = +0.51), and this may explain why the value of R drops from –0.75 to –0.59 in the second table. Second, even if the tables of correlations were based on random numbers instead of observed temperatures, some correlations would appear just by chance. The usual criterion of statistical significance is at the 5% probability level, and this means that, just by chance, 1 in 20 results using random numbers would appear to be significant when in fact they were biologically meaningless.

A third reason for caution in interpreting tables of correlations using meteorological observations is the phenomenon of 'autocorrelation'. This refers to the tendency for temperature in one period to resemble that in a nearby period. The effect can be quite strong, with R up to about +0.8 for adjacent days, and R = c.+0.5 for weather on dates separated by three days (provisional analysis by Stephan Helfer, Senior Mycologist at RBGE, using RBGE meteorological data).

A fourth reason for caution concerns the arbitrary nature of calendar dates. It may be, for instance, that a developmental stage does last about a month, but the start and end dates may not coincide with the start and end dates of a calendar month. If the stage actually lasts from the 15th of one month to the 15th of the next month, then a more biologically meaningful pattern of correlations would emerge if the months used for the analysis started on the 15th of the calendar month.

This last problem can however be solved to some extent by examining the data using different resolutions. Most studies in the past have used monthly average temperatures, but from a biological point of view this is quite arbitrary. In the present study we have tried analyzing the FFDs using temperatures averaged over not only months but also periods lasting three months (seasonal averages), two months, half-months, 10-day periods, quarter-months, weeks and 5-day periods. It happens that the clearest picture for *Galanthus nivalis* emerges using monthly averages, but we know this only because the analysis has been performed at other resolutions. (In general the seasonal and two-month resolutions have not proved useful.)

While correlation simply measures how closely two sets of numbers match each other, regression involves constructing an equation which enables one variable to be predicted from the other one. In the stepwise regression method being developed in this study, the first step involves calculating a regression equation based on one of the strong correlations in the left-hand table, and generally the correlation chosen would be the highest. In this case the JanuaryCY mean temperature has been selected to reflect the fact that there is a reasonably strong correlation for both maximum and minimum temperatures. The relationship has been plotted in the top left graph in Fig. 3. Most of the points fall close to the regression line suggesting that it gives a good explanation of the FFDs, but at least three points are not close to the line, so these are not explained by the January mean temperatures. The equation tells us that the predicted FFD is –6.24 times the January mean temperature plus 48.2 days. If all the points fell on the line, the equation would give an exact prediction of the FFD. The R^2 value of 0.68 means that 68% of the variation in the observed FFDs is explained by the regression equation.

Two points are well above the line, and the vertical distance between the line and the point is called the 'residual'; it is that part of the variation in the FFD that has not been explained by the first regression equation. For the points close to the line, the residuals are very small, and hardly anything is left to be explained. The two points well above the line have residuals of +10.8 days (in 1895) and +17.0 days (in 2002), and the two points furthest below the line have residuals of –13.2 days (in 1894) and –7.7 days (in 2004).

RESOURCE SWITCHING

All twelve residuals from the first regression equation can now be analyzed in the same way as the FFDs were treated. The correlations are shown in the middle table of Table 1. It will be noticed that now some weak positive correlations have appeared, particularly in OctoberPY and NovemberPY. The JunePY negative correlation is still present,

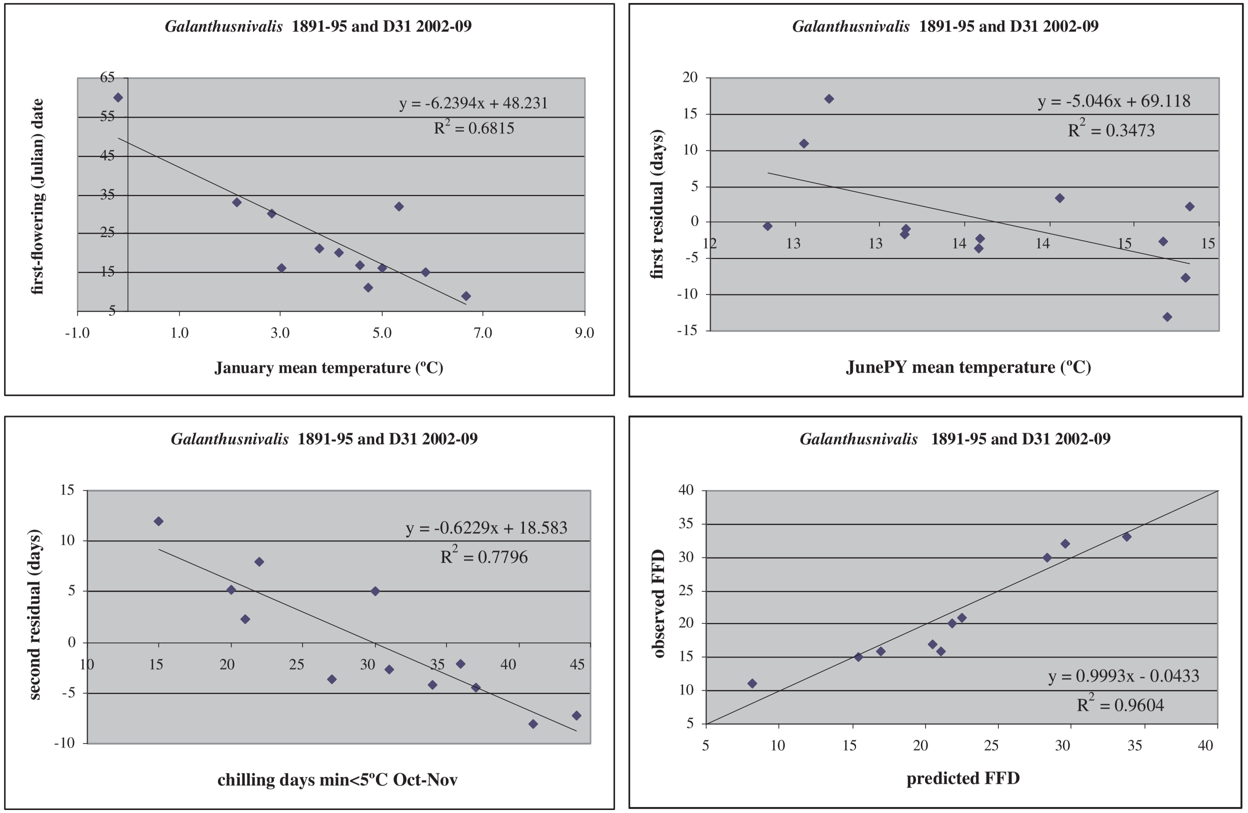

Fig. 3 FFDs and residuals of *Galanthus nivalis* plotted against other variables to illustrate regression analysis.

but smaller than in the left-hand table, as already mentioned. The JanuaryCY mean temperature is shown in bold just to draw attention to the fact that it is the temperature on which the first regression was based, and the first residual (that is the residual from the first regression equation) is bound to be zero.

If it is biologically significant, the JunePY correlation may represent a temperature effect on the early development of the following year's flowers. In snowdrops floral initiation generally occurs from March, when soil temperatures are 3–7°C, and ends in late May (Hartsema, 1961). By June the flower parts are clearly distinguishable within the bulb (Bishop *et al.*, 2001). Since the highest correlation does not appear to coincide with floral initiation, this is presumably not the stage affected. At any stage of development, however, there is the possibility of resource switching, which would involve the plant diverting resources (energy, nutrients and essential chemical compounds) towards certain organs and away from others. During the summer there are many possibilities for such 'house-keeping', and the phenomenon is well known in crop science. Competing 'sinks' for resources are (a) current vegetative growth, (b) next season's vegetative buds, (c) current reproductive growth, such as seed and fruit maturation, (d) next season's reproductive buds, and (e) storage for dormancy and winter. If resource switching is temperature-dependent, it might explain either a positive or a negative correlation between FFD and temperature in any period during which the plant is active. At certain developmental stages, higher temperature may favour the 'parasitic' reproductive buds over the 'host' plant.

CHILLING

The first residuals are plotted against JunePY average mean temperatures in the top right graph in Fig. 3. It is a rather poor correlation (R = 0.59), but it nevertheless explains about 35% of the variation in the first residuals. This graph has its own residuals – the second residuals, derived from the second regression equation – and so the procedure can be repeated to try to explain some of the remaining unexplained variation in the FFDs. The second residuals are correlated with temperatures once more, and the result is shown in the right-hand table in Table 1. Now all of the correlations greater than 0.2 are positive, meaning that higher temperature delays flowering. Since the highest positive values occur in October and November, it is suspected that there is a chilling effect – which we know is true of snowdrops from other sources. So now the second residuals are plotted against the number of chilling days, defined as the number of days in October and November on which the minimum temperature falls below 5°C (bottom left graph in Fig. 3). The correlation coefficient is –0.88, and the regression equation explains 78% of the variation in the second residuals.

Fig. 4 shows that chilling days, defined as days on which minimum daily temperature falls below 5°C, vary from 0 in July to nearly all the days in the month in December and January. In both the 1890s and the years since 2000, the number of chilling days so defined rises rapidly in October and November, and the variation in number of chilling days from year to year is high in those months. If minimum temperatures below 5°C have a chilling effect, it is understandable that October and November are prominent in the analysis, but what is not so clear is whether December and January are also effective for chilling, since there is lower variation in numbers of chilling days from year to year in those months. If there was no variation at all in those months, the analysis would not reveal a chilling effect even if there was one.

MULTIPLE REGRESSION

The stepwise procedure described up to this point has explained the variation in FFDs in terms of the influence of air temperatures at different developmental stages on the way to flowering. The effects of the three factors can be added together to construct a single equation, as follows:

FFD = –6.24 (January mean temperature) + 48.2 days
first residual = –5.05 (JunePY mean temperature) + 69.1 days
second residual = –0.62 (chilldays) + 18.6 days
predicted FFD = –6.24 (JanCY mean temp.) – 5.05 (JunePY mean temp.) – 0.62 (chilldays) + 135.9 days

The stepwise procedure enables us to suggest likely factors influencing FFD, but for technical reasons the quantitative variables indicating the temperature sensitivity of each factor (6.24 days/°C for January mean temperature, 5.05 days/°C for JunePY mean

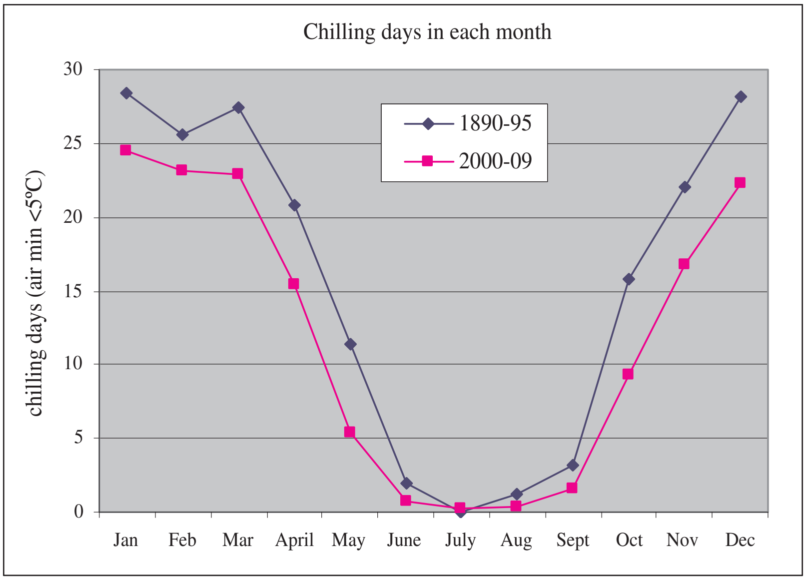

Fig. 4 Graph showing chilling days (days on which daily minimum temperatures <5˚C) in 1890–95 and 2000–09.

temperature, and 0.62 days/chilling day) are unlikely to be biologically meaningful. Moreover the long equation has not been constructed using a legitimate procedure. The selected temperature variables can however now be used in the more complicated calculation of multiple regression, which is not a stepwise procedure. The multiple regression equation produced by Microsoft Excel is:

$$\text{predicted FFD} = -6.88\ (\text{JanCYmean}) - 5.15\ (\text{JunePYmean}) - 0.70\ (\text{chilldays}) + 142.16\ .$$

This is not very different from the equation derived from the simple stepwise procedure, suggesting that the stepwise method is not too misleading. The multiple regression equation, like the stepwise procedure, explains 96% of the variation in FFD. The observed FFD is plotted against the FFD predicted from the multiple-regression equation in the bottom right graph in Fig. 3.

It must be stressed that this is not a demonstration of the actual factors controlling the timing of flowering, but it does demonstrate that the observed FFDs are consistent with the influence of air temperatures at those particular developmental stages. On the basis of these findings we propose the hypothesis that, within the range of conditions experienced during the course of the study, the timing of flowering in *Galanthus nivalis* is largely controlled by air temperatures in January (thermal acceleration), June (influence on an unknown developmental stage), and October/November (chilling days). This hypothesis needs to be tested against further data, as discussed below.

Fig. 5 *Trifolium repens*. Photo: Lynsey Wilson.

PHOTOPERIOD AND FLORAL INITIATION

The snowdrop example has illustrated the effects of temperature on some of the main developmental stages leading to flowering, but another important variable is photoperiod. *Trifolium repens*, White Clover, was selected to investigate possible photoperiod effects, since it is known to be a long-day plant. This means that flower development does not proceed normally until day length becomes longer than a given threshold specific to the genotype of the plant (or, more accurately, until night length has become shorter than a corresponding threshold).

Photoperiod effects pose a problem for this study since day length is independent of the weather, including of course, temperatures. It was initially expected, however, that photoperiod effects might show up in the pattern of thermal acceleration; if thermal acceleration controls the rate of development in the period immediately preceding flowering, and the final developmental stage involving thermal acceleration does not commence until day length has crossed the threshold, then there ought to be a sharp discontinuity at the time when the photoperiod criterion is met.

This has not been borne out by a provisional examination of clover. Unfortunately no data are available from our 19th century records, and the following analysis is based only on 2002–08. Table 2 presents a stepwise analysis of the results from one patch of *Trifolium repens* in the Demonstration Garden at RBGE. While similar patterns emerge from analysis at different resolutions, 5-day periods have been chosen partly in order to demonstrate the utility of such short periods – which, so far as we know, have not been previously used in phenological studies.

The left-hand table shows strong negative correlation between FFD and both maximum and minimum temperatures in 'April-e', which is the fifth 5-day period in the month, in other words 21–25 April. Incidentally there is also quite a strong positive correlation for June-f, long after flowering has begun, and this can presumably be ignored as biologically meaningless for one of the reasons given above for being cautious about interpreting tables of correlations.

When the first residuals are analyzed in the same way, the positive correlations in the left-hand table disappear, suggesting that they were due to chance correlations between the temperatures in April-e and June-f (in fact R = –0.86, which adequately explains the anomalous positive correlation). In the middle table the only strong correlation is for the average mean temperature in April-f. When the procedure is repeated, another fairly strong correlation appears in April-d (see the right-hand table). There is nothing to tell from the table whether the strong negative correlation in March-b is biologically meaningful, but there is in fact a moderately strong relationship (R = +0.76) between the minimum temperatures in March-b and April-d, again suggesting that one or other correlation is an artefact.

The pattern of correlations suggests that temperatures in late April are important in determining FFD. A multiple regression equation using all three temperature variables:

T.repens D28 1997.3698, 2002-08											
				1st residuals from				**2nd residuals** from			
FFDs				FFD / **Apr-e max**				1st residual / **Apr-f mean**			
		R				**R**					
	max	**mean**	**min**		**max**	**mean**	**min**				
Mar-a	-0.75	-0.58	-0.38	Mar-a	-0.47	-0.33	-0.16	Mar-a	-0.65	-0.60	-0.50
Mar-b	-0.44	-0.57	-0.57	Mar-b	-0.41	-0.50	-0.49	Mar-b	-0.69	-0.94	**-0.96**
Mar-c	-0.58	-0.73	-0.74	Mar-c	-0.50	-0.51	-0.42	Mar-c	-0.40	-0.48	-0.46
Mar-d	0.53	0.48	0.34	Mar-d	0.26	0.47	0.65	Mar-d	0.71	0.80	0.78
Mar-e	0.34	0.48	0.56	Mar-e	-0.22	0.15	0.56	Mar-e	0.26	0.43	0.54
Mar-f	-0.33	-0.10	0.28	Mar-f	-0.01	-0.09	-0.13	Mar-f	-0.14	0.27	0.56
Apr-a	-0.04	0.12	0.25	Apr-a	-0.07	0.01	0.09	Apr-a	-0.23	-0.02	0.21
Apr-b	-0.73	-0.70	-0.65	Apr-b	-0.14	-0.19	-0.25	Apr-b	-0.33	-0.31	-0.28
Apr-c	-0.76	-0.66	-0.43	Apr-c	-0.63	-0.49	-0.24	Apr-c	-0.40	-0.12	0.24
Apr-d	-0.78	-0.83	-0.80	Apr-d	-0.38	-0.54	-0.68	Apr-d	-0.54	-0.73	**-0.88**
Apr-e	-0.91	**-0.92**	-0.88	Apr-e	-0.05	**0.00**	0.04	Apr-e	-0.44	-0.53	-0.58
Apr-f	0.35	-0.07	-0.42	Apr-f	-0.50	**-0.87**	-0.83	Apr-f	0.29	**0.00**	-0.27
May-a	0.70	0.69	0.46	May-a	0.25	0.03	-0.30	May-a	0.30	0.31	0.21
May-b	-0.24	-0.50	-0.71	May-b	-0.11	-0.11	-0.09	May-b	0.17	-0.15	-0.50
May-c	0.35	-0.02	-0.28	May-c	0.59	0.46	0.19	May-c	0.73	0.49	0.12
May-d	-0.62	-0.65	-0.67	May-d	0.13	0.08	0.04	May-d	-0.11	-0.23	-0.32
May-e	-0.59	-0.41	-0.22	May-e	-0.14	-0.08	-0.02	May-e	-0.38	-0.54	-0.58
May-f	-0.11	-0.03	0.03	May-f	-0.39	-0.51	-0.53	May-f	-0.06	-0.29	-0.43
Jun-a	-0.23	-0.57	-0.71	Jun-a	-0.40	-0.55	-0.50	Jun-a	-0.08	-0.18	-0.22
Jun-b	0.41	0.14	-0.64	Jun-b	0.02	-0.10	-0.30	Jun-b	0.61	0.46	-0.32
Jun-c	0.18	-0.07	-0.56	Jun-c	-0.07	-0.38	-0.78	Jun-c	0.29	0.02	-0.58
Jun-d	0.66	0.54	0.27	Jun-d	0.01	0.10	0.23	Jun-d	0.12	0.05	-0.07
Jun-e	0.69	0.54	0.34	Jun-e	-0.19	-0.06	0.05	Jun-e	0.31	0.21	0.10
Jun-f	0.65	0.83	0.83	Jun-f	-0.24	0.08	0.35	Jun-f	0.48	0.58	0.57

Table 2 Correlations between first-flowering dates of *Trifolium repens* and 5-day average temperatures, and first and second residuals.

$$\text{FFD} = -1.60\ (\text{Apr-e mean}) - 1.34\ (\text{Apr-f mean}) - 0.90\ (\text{Apr-d min}) + 182.46$$

explains 99% of the variation in FFD. As to be expected, a regression equation using only late April mean temperatures, based on analysis at half-monthly resolution,

$$\text{FFD} = -3.91\ (\text{late-AprilCY mean}) + 185.5$$

also explains most of the variation in FFD (98%). The temperature sensitivities in the first equation (1.60, 1.34, 0.90 days/°C) add up to –3.84; this is close to the –3.91 in the second equation, which is to be expected.

There is a substantial gap between late April and the normal flowering period, suggesting that there is no thermal acceleration of the kind seen in the snowdrops. That this is a significant pattern is supported by the similar result reached by Williams & Abberton (2004). FFD in the clover cultivar S184 was negatively correlated with February and March minimum and maximum temperatures – the time when floral initiation occurs in that cultivar – but not with April temperature; flowering began on 18 May at the earliest. They were also expecting to find evidence of chilling; as in our case chilling may well be needed but would not be expected to show up in analysis if in fact conditions are suitable every year for complete chilling.

A possible explanation of the temperature effect, if it works through floral initiation, might follow from the fact that the critical day length in the photoperiod effect is not always constant. In fact in some clover cultivars it is known to be shorter at lower temperatures (Thomas, 1961). Other things being equal, this would lead one to expect flowering to be earlier when temperatures are low, whereas in our results an earlier FFD is associated with a warmer late April.

A critical day length of 13.5 hours has been found in some clover cultivars, and this day length occurs on 10 April in Edinburgh. It may be, therefore, that we are seeing the effects of temperature, if not on floral initiation, then on the immediately following development at a time when temperature is still limiting. Later, when the weather is generally warmer, temperature may not be a limiting factor, in which case thermal acceleration would not be expected.

As discussed below it would be of interest to know what determines the last-flowering dates in species with a long flowering period. *Trifolium repens* is such a case, since flowering often continues until mid-November. One possibility is that floral initiation is simply dependent on the same day length threshold throughout the year, and the last flowers are initiated just before day length crosses the threshold. Last-flowering dates would then be determined by (a) the date of the threshold (for instance, in Edinburgh a day length of 13.5 hours occurs on 31 August), (b) the development period from initiation to flowering, and (c) the longevity of the inflorescence remaining in flower the longest. Observations are being undertaken to test this idea.

TESTING HYPOTHESES

We are so far not able to use sufficiently large data sets to draw firm conclusions from a statistical analysis. Our hypotheses will have to be tested independently, using further data from the same series, or data from other sites, or experiments.

The ideal situation would be to set up experiments to test specific hypotheses, and a small plant like *Galanthus nivalis* might be suitable, using cloned material in pots. A simple qualitative experiment might involve potted plants kept at one site except for removing designated pots to sites where they would experience warmer or cooler conditions for a set period before being returned to the main collection. If from our hypothesis we expect advancement of FFD when plants are subjected to warmer conditions in January and June, and to chilling days in October and November, but not when subjected to changed temperatures in April–May, August–September, or December; and if we also use replication five (in other words, each treatment to be carried out on five pots), then a set of 65 pots could be prepared, with five subjected to the cold and five subjected to the warm variant of each treatment, with another five as controls. The hypothesis predicts which plants will flower earlier and which will flower later than the control. This might provide material for a student project, and there are many ways in which it could be elaborated into a larger investigation.

ANALYSIS METHODS

There is a limit to what can be achieved using correlation and regression methods on small data sets. The longer that monitoring can be continued – with implications for the staying power of the team of observers, as well as the longevity of the plants – the larger the data set becomes, and the more that can be done with it. There is no reason to think that the current project will not achieve ten years of observations, but twenty or thirty years would be better.

As well as placing on a firmer foundation the kinds of qualitative findings outlined in this paper, with estimates of statistical significance, it should be possible to use regression to measure quantitative properties of the phenomena, such as temperature sensitivities, and more or less exact durations of some developmental stages. Chilling could also be investigated further by trying a wider variety of chilling criteria. For instance, rather than simply defining ‘chilling day’ by an upper temperature threshold, it may be more useful to use an interval, such as $0°C<t<10°C$, where ‘t’ is a chilling temperature. More elaborately, it may be that for a given taxon there is an optimum temperature (say, 7.5°C), and a scale could be constructed weighting temperatures according to their proximity to the optimum. With a much larger data set it might be possible to discriminate between different definitions of chilling requirement. With small data sets it is too easy finding high correlations and regression equations that explain most of the variation in FFD, and results cannot be treated as significant conclusions.

If hypotheses derived from correlation and regression can be tested experimentally on a few species, there might be grounds for drawing up operational definitions of the main developmental stages. For instance significant negative correlations in the period leading up to FFD might be designated ‘thermal acceleration’, and significant positive correlations in November–December might be labelled ‘chilling requirement’. These could be useful in drawing up a functional classification, with the proviso that the labels actually describe merely patterns of correlations, and thorough independent testing would be required to establish that the patterns actually correspond to the implied developmental processes in every case. This might provide numerous opportunities for student projects.

The simple regression approach used so far has been calendar-based, and so may not be suitable for developmental stages which are not tied to the calendar. For instance it has been noticed that snowdrops develop to the stage in which the flower bud bends down to the nodding position, from its original vertical orientation, and there is often a prolonged delay in cold weather before the flower opens once it has nodded. Opening may be triggered by a temperature threshold at about 10°C (Bishop *et al.*, 2001) and after that, flowers may rapidly close and open again according to immediate weather conditions. The stage between nodding and first opening can occur at varying dates, and so this stage may not be detectable using calendar-based correlation. We now measure the ‘nodding date’ as well as FFD. First opening is detected by noting when there is a gap (however small) between the tips of the petals, on the assumption that if a flower opens and closes it cannot close as tightly as before opening. When enough observations have accumulated it may be that nodding will provide a better parameter for a calendar-based analysis than FFD. The date when the first bud passes through the horizontal is also now recorded for *Narcissus pseudonarcissus* and *Eranthis hyemalis*. An equation summarizing the factors controlling FFD in these species may eventually take the form of a stepwise or multiple regression equation with an additional term for the nodding-to-FFD stage linked to a calendar-independent temperature threshold.

Correlation and regression could be adapted to a non-calendar base, for instance by using FFD as the base line and counting periods backwards (such as in Fitter *et al.*, 1995). A more common method is to use measures of thermal acceleration such as growth-degree-days, ‘thermal time’, or heat units, which may be counted not from a fixed calendar date but from the achievement of a temperature, photoperiod or other threshold (as used by Thompson & Clark, 2006). There is scope for combining methods, so that once a large and high-quality data set has been accumulated there are many ways in which useful information about a large variety of plants can be extracted from very simple observations.

A FUNCTIONAL CLASSIFICATION

As already mentioned, one aim of phenological studies at RBGE is to construct a classification of plants into groups which will enable predictions of likely responses to any kind of climate change. This should be useful, given that there is no certainty as to how the Scottish climate is likely to develop in coming decades.

It is already becoming apparent, as pointed out by Williams & Abberton (2004) in relation to clover flowering, that plants may respond to temperature changes in quite short periods while being unresponsive in adjacent periods. This may mean that general predictions such as ‘warmer winter and spring causes earlier flowering and leafing’ do little to advance our understanding of how plants are likely to react. That particular example is in danger of confusing chilling effects and thermal acceleration, which may overlap in duration and to some degree complement each other: less chilling is sometimes associated with a greater thermal-acceleration requirement. In the case of clover the temperature effect may be restricted to a two-week period: in our plants a warmer late April may advance flowering, while change in temperature in early April or early May has little or no effect. Our provisional results suggest that plants’ responses to climate change are going to be complicated and varied, and dependent on responses during a succession of developmental stages of varying and sometimes overlapping durations.

While ultimately it may be possible to construct a ‘natural classification’ based on a few distinct ‘types’ around which most taxa cluster, using as characters many different phenological properties of the plants, in the short term it is more practical to consider constructing an artificial classification using a few measurable properties such as those we are exploring at RBGE. Thermal acceleration and chilling are obvious properties to consider, and we might also consider other developmental stages which are temperature or moisture dependent at particular times of the year. Dormancy setting is a case in point, since interruption of this process may bring about second flowering and also sporadic flowering all through winter.

A further variable which ought to be taken into account is the duration of the flowering period. Many taxa have relatively short flowering periods – perhaps up to two months – which involve the more or less simultaneous flowering of a determinate number of flower buds that have developed over an extended period before flowering (as in the case of many fruit trees and bulbs). The duration of flowering may well depend, in such cases, on temperature and rainfall during flowering, and once every bud has flowered that is an end of it. In contrast there are many species which appear to be able to flower continuously or for extended periods, and some of these were considered to exhibit a ‘mediterranean-type’ of phenology in Sibbaldia 5. Indeed *Arabidopsis thaliana*, *Bellis perennis*, *Cymbalaria muralis*, *Lamium album*, *L. purpureum*, *Sarothamnus scoparius*, *Stellaria media*, *Senecio vulgaris* and *Ulex europaeus* have all been observed flowering throughout the year recently in and around Edinburgh (monitored on every 5, 15 and 25 day or thereabouts of each month). Such ‘population-phenological’ observations (see Sibbaldia 2) are practically useless for discovering what is controlling flowering since, in the case of *Arabidopsis thaliana* and probably other species, the apparently continuous flowering is due to the presence of genotypes with different properties, such that those with a chilling requirement and/or photoperiod requirement flower in certain seasons while others can fill the gaps because their flowering is triggered by different conditions. In the case of taxa with long flowering periods, of which many examples are illustrated in Tomasz Aniśko's interesting book (Aniśko, 2008), it would be necessary to consider the environmental factors controlling the end of flowering, and these may be quite different from those controlling the end of flowering in species with a determinate number of flowers and short flowering period. The last flowering date (LFD) could be investigated using methods similar to those we are developing for FFD.

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