

## LESSONS FROM PHENOLOGY: AN INTERIM REPORT

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### ABSTRACT

Twenty provisional multiple-regression models based on a small data set are presented to account for the timing of first-flower date and other phenological events. Biological mechanisms are suggested to explain the pattern of temperature-dependent developmental stages. The implications for how plants and vegetation are likely to react to climate change are discussed, and attention is drawn to the importance of within-taxon variation in phenological behaviour.

### INTRODUCTION

The Daily Phenology Project at the Royal Botanic Garden Edinburgh (RBGE) began in 2002, following the methods used for monitoring at the Garden in the period from 1850 to 1895. For some plants first-flower dates (FFDs) are now available for nine years, and for a few species last-flower, first-leaf (FLD) and leaf-fall dates have been recorded for a shorter period. Methods of observation are described in earlier issues of this journal.

The multiple-regression method of analysis used in the Daily Project was explained in *Sibbaldia* (Harper *et al.*, 2009). The present article gives further details of high-resolution multiple regression as applied to phenology. While no firm conclusions can be offered as yet using such a small data set, this interim report discusses the kind of results to be expected eventually with a larger data set, and the prospects they offer for furthering our understanding of how plants and vegetation can be expected to react to climate change.

### METHODS

This section on methods explains the models listed in Table 1. It is not essential reading, since the models are summarised in Fig. 2, which is sufficient for understanding the remainder of the article. Readers not interested in the technical details are therefore recommended to proceed immediately to the next section.

‘High-resolution’ refers to three features of the method. While other phenological studies have mostly used monthly average temperatures and observations of large populations, at RBGE we are exploring the use of averages calculated over not only months and longer periods, but also half-months, ten-day periods, quarter-months, and five-day, four-day and three-day periods as well as single days. ‘High-resolution’ also refers to two other features: where possible single plants, in the case of woody species,

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and small patches of herbs are used, rather than large populations spread over large areas; and observations are made daily, as opposed to weekly.

The need for a high-resolution approach is supported by Craufurd & Wheeler's (2009) observation that "in many annual crops, brief episodes of hot temperatures (>32–36°C) can greatly reduce seed set, and hence crop yield, if they coincide with a brief critical period of only 1–3d[ays] around the time of flowering". Temperature effects on day-length sensitivity can also be expected to occur over short periods (Thomas, 1961), and we must keep an open mind on the possibility of other temperature-sensitive developmental stages of short duration.

It is also worth looking for temperature effects over several years before flowering, if only because it is known that some flowers require more than a year to develop after floral (or inflorescence) initiation, for example *Arum*, which may require 18–21 months (Halevy, 1989), and Oil Palm *Elaeis guineensis*, which takes more than 30 months (Hartley, 1967). Pre-initiation stages may also affect flowering times, such as the chilling required – probably for floral initiation – in the second winter before flowering in Olive *Olea europaea* and Peach *Prunus persica* (Therios, 2009; Went, 1961).

Generally three or more accessions of each taxon are monitored, partly in order to detect anomalies – such as one accession behaving abnormally owing to age, disease or damage. Fig. 1 shows the FFDs of six accessions of *Prunus avium* sharing the same accession number (1998.0198 with qualifiers C1 to C6), meaning that they were probably grown from the same seed batch. The individual accession used for initial analysis was chosen to reflect the general behaviour of the taxon; so, for instance, the

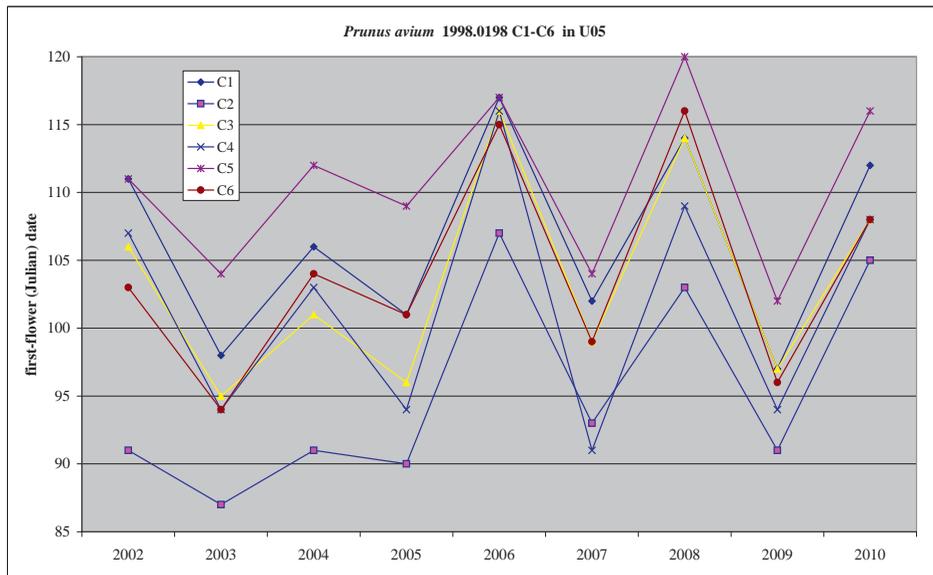


Fig. 1 First-flower dates of six *Prunus avium* trees in bed U05. The Julian date is the day of the year counting from 1 January.

earliest-flowering accession in 2002–2005 is not typical and would be unsuitable for initial analysis. Of course it might be of interest eventually to analyse any untypical accession in order to discover why it behaves differently from the others.

no.	model	n	ind	TINV	t	Rsqu	prob	2002–09					FFD (etc)		2010			obs minus pred (days)	obs minus pred (s.d. units)
								FFD (except where stated)					obs	pred	obs minus pred	obs minus pred	left < right		
			var					av	min	max	range (days)	s.d.			obs minus pred	obs minus pred	left < right		
<i>Alnus glutinosa</i> A02, 1968.7107																			
1	FFD = - 3.04 (earlyDecPYmin) - 0.35 (lateOctPYmean<7deg) - 1.50 (lateJanCYmin) + 53.42	3	2.78	21.5	6.6	0.99	0.000	40.3	32	50	18	5.7	49	50.7	-1.7	8.8	Y	-2	-0.30
<i>Arum italicum</i> D15, 1944.0196B																			
2	FFD = - 7.10 (Dec3PYmean) - 2.33 (JanCYmin) - 2.99 (JulPPYmin) + 209.28	3	2.78	14.6	3.8	0.99	0.000	138.7	128	151	23	8.2	145	145.2	-0.2	6.3	Y	0	-0.02
<i>Arum maculatum</i> D34, 1995.1070E																			
3	FFD = - 8.03 (MarCYmin) - 2.86 (AprCYmax) - 0.57 (NovPYmean<5deg) + 179.99	3	2.78	9.3	6.8	0.99	0.000	117.6	107	126	19	6.6	123	122.9	0.1	5.4	Y	0	0.02
<i>Colchicum autumnale</i> R45, 1969.3927 A																			
4	FFD = - 1.32 (Oct-NovPYmean<7deg) + 3.39 (NovPYmax) + 3.19 (AugCYmean) + 166.27	3	3.18	22.4	6.2	1.00	0.000	228.1	215	237	22	7.7							
<i>Corylus avellana</i> A02, 1903.1005A																			
5	FFD = - 17.80 (lateDecPPYmax) + 98.90	1	2.45	5.2	0.82	0.002		-18.8	-46	21	67	21.9	-49	-51.5	2.5	-30.3	Y	3	0.11
<i>Erythronium dens-canis</i> R20, 1969.4356 A																			
6	FFD = - 5.80 (MarCYmean) - 1.80 (SepPYmean<7deg) + 131.10	2	2.57	7.3	3.5	0.92	0.002	84.875	78	95	17	5.0	95	93.9	1.1	10.1	Y	1	0.22
<i>Fritillaria imperialis</i> 'Lutea', N04/2, 1970.5227 A																			
7	FFD = - 2.67 (lateMarCYmax) - 1.70 (earlyJanCYmean) - 1.73 (earlyJulPPYmin) - 1.08 (lateJanCYmean) + 153.21	4	3.18	33.1	23.1	0.99	0.000	95.1	84	104	20	5.8	103	103.6	-0.6	7.9	Y	-1	-0.10
<i>Galanthus plicatus</i> R17, 1978.1807A																			
8	FFD = - 2.94 (earlyDecPYmax) - 0.88 (earlyFebCYmax) - 0.21 (Sep-OctPYmin<5deg) + 63.37	3	2.78	8.0	3.0	0.96	0.003	37.9	33	47	14	5.0	43	46.2	-3.2	5.1	Y	-3	-0.64
<i>Lathyrus vernus</i> R26, 1958.1961 A																			
9	FFD = - 5.73 (lateFeb-lateMarCYmax) - 1.59 (earlyFebCYmean<10deg) + 166.93	2	2.57	11.4	4.0	0.97	0.000	91.6	81	104	23	7.2	101	100.0	1.0	9.4	Y	1	0.14
<i>Leucojum vernum</i> D03/22, 1937.0520A																			
10	FFD = 10.43 (Dec3PYmax) + 4.50 (OctPYmin) - 6.76 (JanCYmax) + 4.48 (Sep-NovPYmax) - 81.24	4	3.18	30.6	21.1	1.00	0.000	34.8	13.0	52.0	39	14.2	65	61.5	3.5	30.3	Y	4	0.25
<i>Malus baccata</i> 'Hiemalis', S20, 1903.1006																			
11	FFD = - 5.71 (lateFeb-lateMarCYmax) + 158.41	1	2.45	7.6	0.91	0.000		104.1	97	119	22	6.9	116	113.9	2.1	11.9	Y	2	0.30
<i>Malus sieboldii</i> FLD, A01, 1908.1055A																			
12	FLD = - 9.98 (MarCYmin) - 5.54 (FebCYmax) + 5.81 (JunPYmean) + 70.73	3	3.18	8.2	4.9	0.98	0.005	79.4	71	99	28	9.5	96	100.2	-4.2	16.6	Y	-4	-0.44
<i>Malus sieboldii</i> FFD, A01, 1908.1055A																			
13	FFD = - 5.45 (AprCY-A-mean) - 1.43 (MarCY-C-D-min) - 2.34 (JunPY-A-min) - 0.62 (JanCY-C-mean) + 170.74	3	2.78	49.8	20.1	1.00	0.000	94.1	83	111	28	8.0	114	103.8	10.2	19.9	Y	10	1.27
<i>Oxalis acetosella</i> U07, 2003.0016B																			
14	FFD = - 11.14 (MarCYmin) - 1.1 (Nov-DecPYmax<7deg) + 150.31	2	2.57	5.2	4.5	0.89	0.004	100.6	87	113	26	9.0	103	100.9	2.1	2.4	Y	2	0.23
<i>Prunus cerasifera</i> A01, 1969.8277																			
15	FFD = - 6.49 (earlyJanCYmean) - 1.02 (lateOctPYmean<10deg) - 1.64 (earlyFebCYmin) + 91.73	3	2.78	17.3	8.1	0.99	0.000	48.6	27	61	34	12.8	87	87.7	-0.7	38.4	Y	-1	-0.05
<i>Rhododendron yunnanense</i> M18, 1969.8644A																			
16	FFD = - 4.02 (lateFeb-lateAprCYmean) + 154.29	1	2.45	7.3	0.71	0.009		125.5	120	131	11	4.4	130	129.8	0.2	4.5	Y	0	0.05
17	FFD = - 4.27 (lateFeb-lateAprCYmean) - 1.0 (DecPYmax<5deg) + 161.57	2	2.57	30.2	10.1	0.93	0.001	125.5	120	131	11	4.4	130	118.6	11.4	4.5	N	11	2.61
<i>Scopolia carniolica</i> W21, 1964.2567																			
18	FFD = + 5.85 (lateOct-earlyNovPYmin) - 0.85 (earlyJanCYmax) - 1.79 (lateJanCYmax) + 64.06	3	2.78	16.9	3.5	0.99	0.000	75.0	68	89	21	7.3	85	83.0	2.0	10.0	Y	2	0.28
<i>Trifolium repens</i> D28, 1997.3698																			
19	FFD = - 3.51 (Apr-d-f-CYmean) + 0.37 (25 MarCYmax) + 177.56	2	2.57	40.4	10.1	1.00	0.000	148.5	141	156	15	4.4							
<i>Rana temporaria</i> FSD, D35 pond																			
20	FSD = - 2.60 (Feb28-Mar7min) + 0.52 (Feb15-17min) + 76.09	2	2.57	30.2	10.1	1.00	0.000	72.8	64	85	21	6.4	78	78.4	-0.4	5.3	Y	0	-0.06

Table 1 Regression models based on eight years of observations (2002–2009) with predictions of 2010 dates compared with observed 2010 dates. FLD = first-leaf date (model 12); FSD = frog/spawn date (model 20). For further explanation see 'Methods'. Model 12 is based on 2003–2009 observations.

Table 1 presents 20 models, numbered in the left-hand column. The analysis has been carried out in Microsoft Excel. A typical entry comprises three lines, the first giving the species or other taxon name, the bed number and the accession number. The second line gives the model in the form 'FFD =  $m_1x_1 + m_2x_2 + \dots + \text{constant}$ '. In each case  $x_1$ ,  $x_2$ , and so on are independent temperature variables, either an average calculated over a period (occasionally a value for a single day) or a number of chilling days. The values  $m_1$ ,  $m_2$  are regression coefficients. Years, counting backwards, are CY (current year, or latest year in which flowering occurs), PY (previous year), 2PY, 3PY and so on. 'Min', 'mean' and 'max' refer to the daily observations of minimum and maximum temperatures at the RBGE meteorological station on the north side of the Edinburgh Garden, and the daily mean as calculated from the maximum and minimum readings. Monthly averages are in the form 'MarCYmin' (model 3), two-month averages in the form 'Sep–NovPYmax' (model 10), and half-month averages in the form 'lateJan–CYmin' (model 1). For shorter periods, ten-day averages use 'I-II-III' to represent thirds of a month, quarter-month periods are distinguished by 'A-B-C-D' (model 13), six-day periods by 'a-b-c-d-e-f' (model 19) and ad hoc periods by 'Feb15-17min' (model 20). Presumed chilling periods are measured as the number of days in the period in which the maximum, mean or minimum temperature is below 10, 7 or 5°C. These nine ways of measuring chilling are expressed in the form 'SepPYmean<7deg' (model 6).

The third line in each entry gives statistics relating to the 2002–2009 model and its success in predicting the 2010 FFD. The second column shows the number of independent variables or 'mx' terms in the model ('n ind var' in the column heading). TINV is a quantity relating to the t-test, and is a function of 0.05 probability and the number of degrees of freedom. The t values for the independent variables (equal to the regression coefficient divided by the standard error), listed in the order they occur in the model, should be larger – and if possible much larger – than the TINV value. All t values in the table are larger than the relevant TINV value, but often not large enough for a satisfactory result. 'Rsq' is the proportion of variance in FFD explained by the model: thus model 1 accounts for 99 per cent of the variance in FFD in 2002–2009. 'Prob' is the probability that the result could have occurred by chance, as calculated from the F statistic.

The next five columns present the average (av), minimum (min) and maximum (max) FFD in 2002–2009, and also the range (difference in days between max and min) and standard deviation (s.d.), which is a measure of the scatter of the values around the average. For 2010 are given the observed value (obs) and the value predicted from the model (pred). The next three columns show whether the predicted value is closer than the average to the observed FFD, and this test may indicate the predictive performance of the model. In all except one case the result is Y (yes). Finally, the last two columns repeat the absolute difference in days between observed and predicted values, this time to the nearest day, and the same quantity expressed in standard-deviation units, which enables a better comparison to be made between species with small and large ranges in FFD values. These informal tests are not expected to be useful in all years; for instance

in some years all three values – average, predicted and observed – will be close together and it will not be possible to tell whether ‘pred’ is significantly closer than ‘av’ to the observed value. (Data for 2010 are missing for model 4 and model 19 because flowering had not occurred at the time of writing.)

Observational errors are estimated to range from one day – to allow for the fact that a plant may come into flower after monitoring on a given day, but will be recorded only for the following day when it is first seen – to several days in the case of plants which come into flower slowly or reversibly, or where the flower is small in relation to the size of the plant and/or inconspicuous and difficult to see. In the last two columns the values 2 or fewer days and 0.25 or fewer standard-deviation units are in bold, merely to draw attention to them.

With such a small data set for each plant, formal tests of significance are not appropriate, and instead the statistics shown in the table are used to guide the construction of models, which at this stage are treated as hypotheses explaining the timing of flowering. The ideal model would be one that functions well as both a ‘descriptor’ – describing existing data (2002–2009) – and a ‘predictor’ – predicting future FFDs.

A good descriptor should have high values of  $t$  and  $R^2$  in the table, and a low value of prob. Other things being equal, a model with only one or two independent variables is preferable to one with many independent variables, and a model that makes biological sense is preferable to one that does not. Thus there has been a bias towards models that can be interpreted in terms of well-known biological processes (listed in the next section). If a plant in fact has more than two temperature-sensitive developmental stages these are unlikely to be demonstrated clearly at this early stage in the study.

A good predictor is simply a model that yields an accurate prediction, bearing in mind observational errors. Any prediction within about two days of the observed FFD can therefore be regarded as accurate, and smaller values of ‘obs minus pred’ carry no extra significance. It is encouraging that the data set is already large enough for some models to provide accurate predictions.

Early 2010 was in one sense a frustrating time for the study, on account of the unusually cold winter and spring. This may be why some good descriptor models performed poorly as predictors of 2010 FFDs. A case in point is *Rhododendron yunnanense*. A good prediction is given by model 16, in which the single independent variable is average mean temperature in the period late Feb–late Apr just before flowering. However it explains only 71 per cent of the variation in FFDs in 2002–2009, and in particular it fails to account for the more or less steadily increasing FFD from 2002 to 2006. This increasing lateness of flowering is likely to be the result of incomplete chilling owing to warming winters – supported by model 17, which includes a chilling effect in December. Ninety-three per cent of variation in FFD is now explained, but the model fails to predict since it leads one to expect very early flowering in 2010, not the late flowering that actually occurred.

A possible explanation concerns the omission of early-May temperatures, if temperatures in that period had an effect in 2010. Since December 2009 and early 2010

have extended the range of temperatures experienced during the study, a model based on 2002–10 will take this wider range of conditions into account and may prove to be both a better descriptor and a better predictor. The study must be continued for a few more years at least before we can have confidence in the method and in the resulting models.

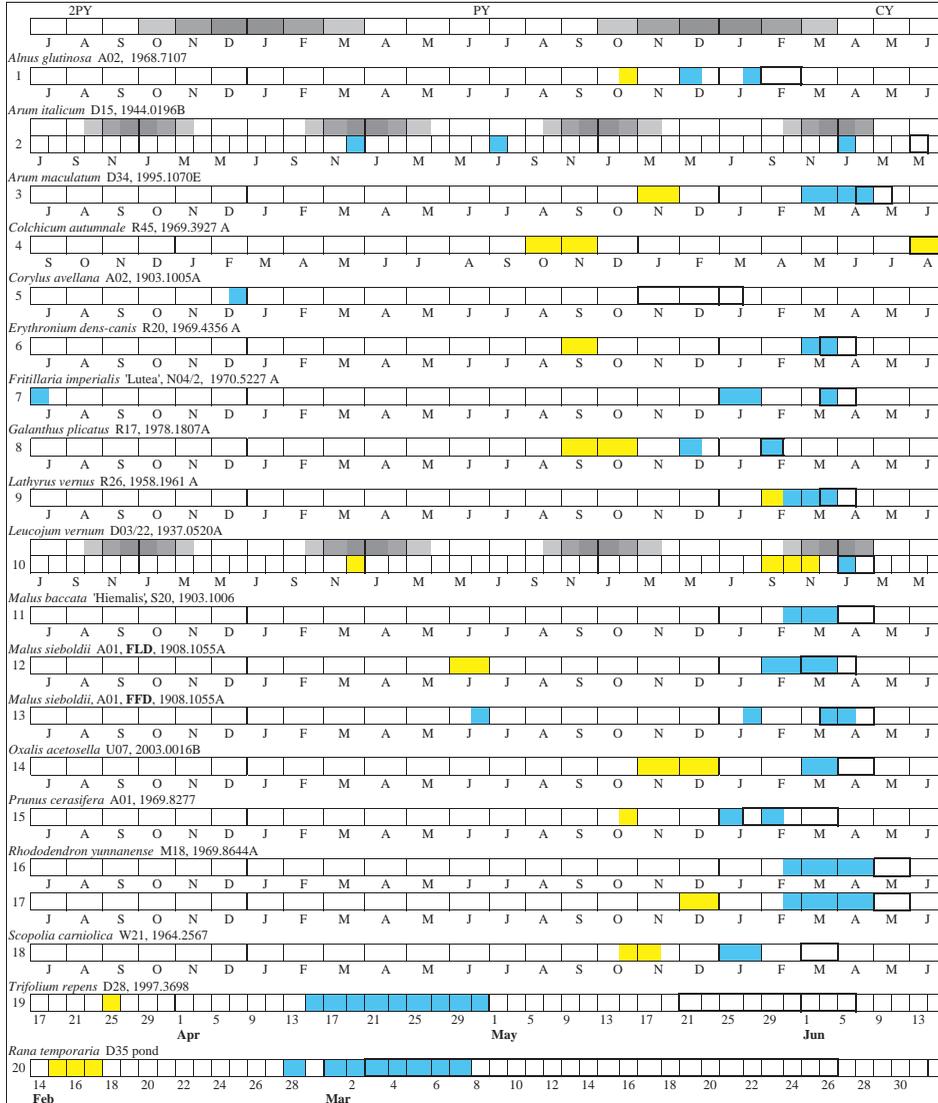


Fig. 2 Timeline representations of the models in Table 1. FFDs occur in the bold-outlined periods. Blue represents negative correlation between temperatures in the marked period(s) and FFD (thermal acceleration) and yellow represents positive correlation (thermal deceleration). The bar with grey cells at the top of the diagram symbolises the varying day lengths of the seasons in most of the timelines, covering two years, while model 2 and model 10, covering four years, have their own bar. Model 22 and model 23 have individual time lines.

One problem with time-series data is that multiple-regression analysis is applicable only if the FFDs and temperatures of one year are independent of other years. If independence cannot be established for the data a more sophisticated method of analysis will have to be found incorporating a temporal-dependence structure between the observations (or residuals) in the model (Zuur *et al.*, 2009).

#### INTERPRETATION OF THE MODELS

Fig. 2 shows the models of Table 1 as timelines, in the same sequence as in the table. The independent variables of the models are shown as coloured periods, blue representing those in which higher temperatures are associated with earlier flowering (thermal acceleration). Yellow areas represent periods of thermal deceleration, in which higher temperatures are associated with later flowering. FFDs occur in the bold-outlined boxes.

The terms ‘thermal acceleration’ and ‘thermal deceleration’ are operationally defined, in other words they refer merely to the existence of periods in which there is a significant correlation between temperature and FFD. If these models or models of similar form can eventually be established with confidence – that is, based on an expanded data set, or tested against independent data sets or experimentally – they will be sufficient to make predictions about how plants will respond to climate change, in so far as temperature is involved. An important qualification is that such predictions are expected to be useful only within a limited range of climate change, corresponding to the range of conditions experienced during the study. (The same analysis could easily incorporate non-temperature variables, such as sunshine hours or precipitation, but this has not so far been tried.)

Assuming that thermal acceleration and deceleration can be demonstrated satisfactorily, the results would be of considerably greater biological interest if explanations can be found for the temperature effects. Before listing possible explanations of acceleration and deceleration, a general working assumption needs to be made clear: it is assumed that a sequence of developmental stages leads up to flowering (or leafing, etc), some of which are temperature-sensitive and some of which either are not temperature-sensitive or are, but with different sensitivity. Moreover, it is assumed that these developmental stages are generally in sequence, although some may overlap when considered over periods of a week or more – for instance if chilling occurs during the nights and acceleration of development during daytime. A further assumption is that these stages are tied to the calendar – although it is by no means obvious why this should be true: if a given stage occurs on different dates each year, the analysis would be unlikely to reveal them. On the other hand, if satisfactory models are found, that is ones which are both good descriptors and good predictors, it can perhaps be assumed that developmental stages are in fact fairly closely tied to the calendar, as might be expected if there is occasional photoperiod-based calibration and/or some other time-keeping mechanism within the plant. In the case of thermal acceleration of development in the period leading up to flowering, where there is a variable FFD there will also be a temperature-sensitive period

that is not closely tied to the calendar, at least as regards its end date. In such cases it may be necessary to adapt the analysis to accommodate ‘thermal time’ (for example degree-days) or some other variable independent of the calendar.

#### *Thermal acceleration*

This is probably most commonly explained, especially in spring, by the speeding up of chemical reactions in warmer conditions when temperatures are below the optimum. A special case could involve chilling (in which temperatures need to fall below an upper threshold for development to proceed optimally): if chilling in fact occurs only in a narrow range of temperatures – say, 0–10°C – then if temperatures are hovering around the lower threshold warmer conditions would make flowering happen earlier because chilling is occurring more strongly. Another explanation can be illustrated by the grapevine. Axillary buds occur in groups of three: the primary buds are the least winter-hardy and have the largest inflorescence primordial, the tertiary buds are the most winter-hardy and generally have no inflorescence, and the secondary buds are intermediate in both respects. Following a mild winter the primary buds will develop and produce abundant flowers, in a more severe winter the secondary buds will develop and produce fewer flowers, possibly beginning flowering later than the primary buds would have, and a very severe winter may prevent flowering altogether while permitting vegetative growth. So we have the likelihood that lower temperatures during winter will be associated with later or absent flowering on account of differential mortality between the different kinds of bud (Creasy & Creasy, 2009).

#### *Thermal deceleration*

This also has a number of possible mechanisms. One is the converse of the first mechanism given for acceleration: if temperatures are above the optimum for a given chemical process, higher temperatures can be expected to slow development. A common mechanism is chilling: deceleration would occur when temperatures are hovering around the upper threshold. Another explanation would involve resource switching (also known as carbohydrate partitioning): if, during summer, higher temperatures favour seed and fruit maturation over development of the following year’s reproductive structures, development of the latter will be retarded and flowering will probably be later. It is known that photoperiod thresholds are sometimes temperature-dependent, with cooler conditions being associated with shorter day-length requirements (Thomas, 1961), and this might give rise to thermal deceleration for a short period around the time of the day-length threshold. Late-summer-flowering species may be stimulated to flower by low temperatures and/or increased moisture in the soil, as possibly seen in *Colchicum autumnale* (model 4). Finally, leaf fall can be accelerated by frost. There may well be other mechanisms causing thermal deceleration in phenological events.

Some developmental stages may be initiated by thresholds. Photoperiod has already been mentioned, and this will probably be strictly calendar-dependent when it is not temperature-sensitive. Other thresholds might be the achievement of a particular temperature, such as c. 10°C for the opening of *Galanthus* flowers (Bishop *et al.*, 2001), or the completion of chilling, neither of which is tied to the calendar but both of which are temperature-sensitive. These thresholds offer further possibilities for interpreting the models and, unlike most of the mechanisms listed above under ‘thermal acceleration’ and ‘deceleration’, they may produce qualitative rather than quantitative effects. Explaining the models, if they are shown to be reliable, will involve further work, and the present study is concerned merely to produce reliable models.

#### PATTERNS REVEALED BY THE MODELS

On the basis of models representing many species, it is hoped eventually to lay the foundations of a functional phenological classification, involving the construction of groups of plants that can be expected to respond to climate change in similar ways. The simplest such group would include plants that are not expected to respond to climate change at all, such as those with a temperature-insensitive photoperiod requirement and which do not have any temperature-sensitive developmental stage before flowering. No such plant has yet been found, but one may turn up.

The next simplest group might include plants that have just one thermal-acceleration stage, for example *Corylus avellana* (hazel) (model 5) and *Malus baccata* (model 11). The latter involves thermal acceleration shortly before flowering, while hazel appears to respond most strongly to winter temperatures a year before flowering. In both cases warmer winters can be expected to result in earlier flowering. We cannot conclude that these species do not have a chilling requirement, since this would not be revealed by the analysis if in fact the chilling requirement were fully satisfied every year; however, a chilling requirement may become evident if conditions become so warm that chilling is sometimes not completed.

In a few species there appear to be one or more additional periods of thermal acceleration – for instance *Fritillaria imperialis* (model 7) and *Malus sieboldii* (model 13). In the latter case the late-June effect may be related to floral initiation, and this could conceivably also be the explanation for the July2PY effect in *F. imperialis*. Without further evidence one can only speculate at this stage, and of course the models may prove not to be robust as further data are collected.

Many spring-flowering species have periods of thermal deceleration in autumn or winter, and these are provisionally interpreted as chilling requirements. In *Erythronium dens-canis* it appears to occur as early as September (model 6). Chilling more often occurs in October (model 1, model 15) or November (model 3) or both (equus 4, 18). In *Oxalis acetosella* (model 14) it is in November–December, and in *Rhododendron yunnanense* (model 17) in December. *Leucojum vernalis* (model 10) may have two chilling periods, in September–November immediately before flowering, and also in

December3PY. (This result should be treated with caution, despite the reasonably good descriptive properties of the model: a major source of error using a small data set is chance correlations between temperatures in non-adjacent periods. This problem should become less serious as the data set expands.)

It has to be admitted that reading a chilling effect into a period of thermal deceleration is a somewhat hazardous undertaking. While the mostly short periods may mean that chilling is often completed quickly, at various times from September to December, there is the possibility that these results are due to the pattern of variation in temperatures, in particular their wide variation in autumn and the much smaller variation in mid winter. If, for instance, the daily minimum temperature is below 10°C for 31 days in every December, analysis relying on correlations using a  $\text{min} < 10^\circ\text{C}$  criterion will not reveal any chilling in December. With a larger data set it may be possible to distinguish biologically meaningful results from these artefacts due to temperature variation, and meanwhile we need to keep an open mind on how to interpret autumn and winter thermal deceleration.

Other examples of thermal deceleration are probably not due to chilling. A case in point is *Trifolium repens* (model 19). A model based on 2002–2008 and using only the thermal acceleration in late April gave an accurate prediction of FFD in 2009. Analysis of the 2002–2009 data set suggests in addition a brief period of thermal deceleration on or around 25 March. While this could conceivably be a chilling effect, it is more likely to be a manifestation of the temperature dependence of a photoperiod threshold, as already mentioned; 25 March is shortly after the spring equinox, and corresponds to a daylength at the latitude of Edinburgh of about 12.5 hours. If this explanation is plausible, it might also be applicable to *Lathyrus vernus* (model 9), which appears to have a short period of thermal deceleration in early February. This is perhaps the cue to introduce RBGE's 'honorary plant' – the Common Frog *Rana temporaria* (model 20). The appearance of frogs and/or spawn at the pond in the Demonstration Garden has been recorded since 2002, and the model for FSD (frog/spawn date) was, ironically perhaps, the first one to be found that fulfilled the requirements of being both a good descriptor and a good predictor. The 2002–2009 model gave an exact prediction of FSD in 2010, and it contains a brief period of thermal deceleration from 15 to 17 February. The immediate impulse was to proclaim the discovery of a chilling requirement in frogs, but a temperature-sensitive photoperiod requirement is another possibility, assuming that Edinburgh's frogs are exposed to daylight at that time of the year.

Quite a different example of thermal deceleration is that shown by *Malus sieboldii* first-leaf date (FLD: model 12). Chilling is presumably not relevant to June, and the most likely explanation is that resource switching is occurring, such that in warmer weather carbohydrates and other resources are being diverted from growth of the following year's foliage into some other part of the plant, such as seed and fruit, or the following year's reproductive tissues.

If the patterns revealed by the models can be demonstrated convincingly, they will raise all manner of interesting biological questions. For instance, are the frequently

rather short and distant periods of acceleration and deceleration actually separated by periods when development is really not temperature-dependent? In some cases this may be in doubt, since the problem of auto-correlation – the tendency of adjacent periods of weather to be similar, such as one day and the following day – means that the boundaries of temperature-dependent periods cannot be exactly determined: the periods may be longer or shorter than suggested by the models. However, the temperature independence from 18 to 27 February in frogs (model 20) and from 26 March to 13 April and from 1 to 20 May in *Trifolium repens* (model 19) can almost certainly not be explained away by auto-correlation.

#### IMPLICATIONS FOR PLANTS, VEGETATION AND CLIMATE CHANGE

It seems clear that there is considerable variation between species regarding their expected responses to moderate climate change. This is evident from the differences in the three species depicted in the graphs (Figs 1, 3 and 4). Even if we consider only the well-known processes of chilling and thermal acceleration of development, it appears that both occur at different times, for varying durations and with varying temperature sensitivities (measured by the regression coefficients). To take the simplest climate-change scenario, uniform warming would lead species with chilling and thermal acceleration of development to react differently if only on account of the balance of the two processes. In the first few years of the study some plants advanced their FFDs fairly steadily – such as *Corydalis solida*, *Corylus avellana*, *Crataegus monogyna*, *Eranthis hyemalis*, *Leucojum vernum*, *Malus sieboldii*, *Prunus cerasifera*, *Ranunculus*



Fig. 3 Different phenological behaviour in three accessions of *Alnus glutinosa*.

*ficaria* – while others exhibited the opposite tendency – *Colchicum autumnale*, *Malus baccata* (from 2003 to 2006), *Oxalis acetosella*, *Rhododendron yunnanense*, *Scopolia carniolica*, *Trifolium pratense*. To take one example from each group, *Prunus cerasifera* (model 15) has stronger thermal acceleration in January and February than chilling in October, while *Scopolia carniolica* (model 18) appears to have a strong chilling temperature sensitivity in late October and early November.

It is not only species – and sometimes closely related species – that show diversity in their responses to temperatures. Fig. 3 shows the FFDs of three alder trees *Alnus glutinosa*. The upper two graphs are from young trees, probably from the same seed batch, and they clearly have similar behaviour although one flowers consistently later than the other. The lowest graph is from an older tree in another part of the Edinburgh Garden. Although it is possible that the differences between the lower two graphs are merely phenotypic and due to different environments and/or histories, the dissimilar behaviour is typical of what is expected from genetic differences within a species. The near-coincidence of FFD in 2002 but lack of coincidence in other years suggest that different factors are responsible in the two trees for the timing of flowering. To take two other well-known examples, both *Trifolium repens* and *Arabidopsis thaliana* show considerable intra-specific variation in phenological behaviour. So we must be cautious about generalising to a whole species the findings based on a single individual – or, for that matter, based on a population assumed to be homogeneous when the within-population variation has not been investigated.

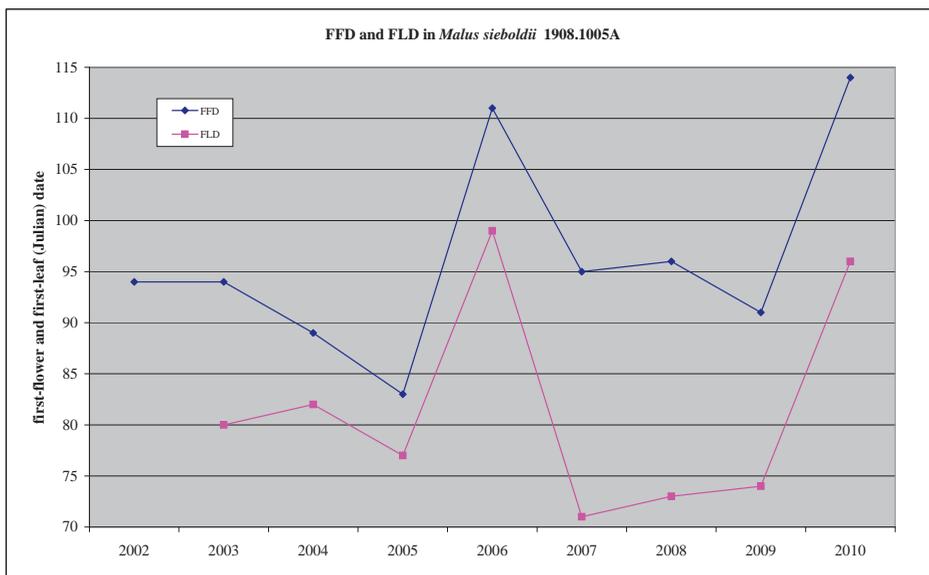


Fig. 4 Different phenological behaviour of first-leaf date (FLD) and first-flower date (FFD) in *Malus sieboldii*. FLD is earlier than FFD by six and twenty-four days in 2005 and 2007 respectively. (FLD was not being measured in 2002.)

We can go further and note that different parts of the same individual plant may behave differently in a phenological sense. Fig. 4 shows FFD and FLD in a *Malus sieboldii* tree. While in some years the two events were behaving similarly, the difference in days between FFD and FLD is far from consistent, suggesting that the reproductive and vegetative parts of the plant are reacting to different factors. No single model will account for both FLD and FFD, and models 12 and 13 are the best that have so far been found for FLD and FFD respectively. Neither model is particularly convincing, but together they suggest that in June of the previous year higher temperatures favour floral development while retarding development of the vegetative buds – a possible example of resource switching, as already mentioned.



Fig. 5a & b *Scopolia carniolica*: in var. *carniolica* (1964.2567), stem elongation and leaf expansion are well advanced before the pendent flower buds open, while in var. *brevifolia* (1959.2373A) flowers open relatively much earlier, before stem and petioles have elongated and the buds have become pendent. Photos: a: Christine Thompson; b: Geoffrey Harper.

Another striking demonstration of the independence of vegetative and reproductive organs is seen in *Scopolia carniolica*. The three accessions being monitored at RBGE are arranged on a spectrum of relative timing of vegetative and reproductive phenology. At one extreme (Fig. 5a) the vegetative structures are well advanced before flower buds emerge, become pendent and eventually open, while at the other extreme (Fig. 5b) flowering occurs at a much earlier vegetative stage. In *Hacquetia epipactis* the effect can be even more extreme, with flowers in some varieties opening almost before the plant has emerged above ground level.



Fig. 6 *Corylus avellana*: flowering in November 2004 (1903.1005A). Photo: Geoffrey Harper.

Such dysfunctional timing can have ecological consequences. For instance, hazel *Corylus avellana* (Fig. 6) will sometimes flower as early as November, before the leaves have fallen. Clearly the factors controlling leaf fall are not the same as those controlling flowering, even though both events can occur at about the same time. If this exceptionally early flowering in hazel were to become frequent, as a consequence of warmer winters (model 5), it might reduce transfer of genes between plants and therefore outbreeding, and so affect the ability of the species to adapt to changing conditions.

The implications of climate change for vegetation are even more complex than for individual plants or species. Some oft-quoted examples of mismatches – in flowering and pollinator availability, fruiting and dispersor availability, and caterpillar availability when migrant birds arrive and nest – illustrate the general point that if species react differently to climate change their mutual adjustments may be disrupted, with serious consequences for the competitive ability or even survival of some populations. Körner (2006) has cited the interesting example of nutrient loss in some soils: if micro-organisms remain active during warmer winters while temperatures are still not high enough for the roots of vascular plants to absorb released nutrients, they may be lost through leaching. Nutrient impoverishment of the soil could bring far-reaching changes in the composition and structure of natural communities.

Much attention has been paid to migration rates of species towards higher latitudes and altitudes, with the implication that climate change may be occurring too rapidly for some trees and other plants to adjust their ranges fast enough. For this and other reasons we should be prepared for some kinds of vegetation to disappear, and perhaps for other kinds to arise. The steppe-tundra (or tundra-steppe) is a case in point (Troeva *et al.*, 2010): this community was widespread over much of Europe and northern Asia in the later stages of the last glaciation, and now has largely disappeared from the world. If new combinations of geographical and climatic conditions arise, as they might in the current period of climate change, new kinds of vegetation can be expected to appear as others disappear.

#### CONCLUSIONS

The main conclusion of this interim report is that interesting results are beginning to emerge and that it is worth continuing the study. The approach seems to be justified on general biological grounds, as well as in the results so far achieved.

If temperature-sensitive developmental stages can be identified using high-resolution multiple regression, it is hoped that the results can be tested against independent data sets and the work taken further experimentally, in particular by the use of growth chambers simulating different weather patterns. In this way results that take many years to accumulate using established outdoor collections in botanic gardens, relying on natural weather variation from year to year, could be assembled within one or a few seasons in controlled conditions.

Sadly the methods used in this study make it something of a 'race against time'. If at least ten years are needed to acquire a satisfactory data set, the question arises as to whether the plants under observation – let alone the observers – will last that long. Mortality, disease, damage and redevelopment of the Edinburgh Garden are some of the causes of plants being lost from the study. Anyone planning this kind of work should allow for considerable wastage, and it is always a matter of chance whether the really interesting plants will stay the course. Fortunately RBGE is an excellent site for such a study, and prospects are good that something valuable will come out of it in the next few years.

One advantage of this 'bottom-up' approach is that the intra- and inter-taxon variation can be assessed before grouping plants in order to construct larger data sets or to make generalisations. One aim in this study is to construct a phenological classification of plants, and it is already clear that taxonomic groups cannot be assumed to have uniform phenological behaviour. Indeed within-species variation is considerable in some species, so that generalising at this stage about how familiar groups of plants are likely to react to climate change – such as 'grasses', 'trees' or 'spring-flowering plants' – is likely to be highly misleading.

The general impression gained so far is that we must expect the responses of plants and vegetation to climate change to be extremely complex. Multiple regression provides

a means of assessing the likely responses of individual plants, species and species groups to any kind of moderate climate change, and botanic gardens are ideal places to carry out this work. It is recommended that the high-resolution methods of observation being developed at RBGE – described here and in Harper *et al.* (2009) – should be adopted in many other botanic gardens and similar institutions.

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