

# **Tracking phenological shifts and evolutionary impacts related to climate change**

**Phyllis Frances MacGillivray**

School of Earth and Environmental Sciences  
University of Adelaide

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*Sakura* (cherry blossom)

Recordings of memorable phenological events, such as the flowering of cherry trees in Japan, represent the oldest known phenological series with records dating back to the 9th Century. This display may have retained some of its cultural and religious significance, but now attracts additional economic benefits from tourism. Photography: Asa Fujimoto.



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## Thesis Summary

Phenology is the study of recurring life-cycle events that are initiated and driven by environmental factors, such as the response of flowering time to the prevailing climate. Ongoing climate change is thus expected to impact on the flowering time of plant populations with consequences for reproductive success in the short term and their survival in the long term, along with potentially widespread repercussions for associated ecological health and function. Tracking phenological shifts in response to past climate variability provides a benchmark or reference point for gauging future impacts.

The introductory chapter of this thesis presents a review of the literature as it relates to my research documented in the following three chapters. Chapter 2 provides an exploration of the impacts of climate on the flowering phenology of the South Australian endemic *Diuris* orchid genus. A statistical analysis, trialling the suitability of Generalized Additive Models for Location, Scale and Shape (GAMLSS) for modelling of a long-term, historical dataset showed a significant curvilinear trend, with peak flowering advancing over time. This investigation was extended to determine the main and interactive effects of temperature and rainfall as specific drivers of *Diuris* flowering phenology (Chapter 3). A highly significant flowering response to seasonal temperatures and rainfall was identified, with shifts to earlier flowering in warmer and drier seasons expected under climate change scenarios.

Chapter 4 comprises various analyses of a 44-year replicate data set of 112 *Pyrus* (pear) trees growing at the University of Adelaide Waite Arboretum. This aspect of my research provided a unique opportunity to study the phenological responses of a non-native genus at the species and individual levels, when subjected to identical environmental conditions. A general response to minimum temperature was, on occasions, overridden by an early flowering response initiated by drought-breaking rains. This study also allowed a comparison to be made between *Pyrus* phenology in the northern and southern

hemispheres, and an insight into the potential economic impacts for South Australian horticulture.

Evolutionary implications for all study species arising from climatically-induced phenological shifts are outlined in Chapter 5, including a consideration of the likelihood that the rate of evolutionary change will be sufficient to keep pace with predicted climate change scenarios. Findings from these investigations are then considered in relation to the selection of bioclimatic indicators. In this sixth chapter, I challenge the validity of many assertions and assumptions presented in the literature. This thesis concludes that the stresses of ongoing climate change will have a selective impact on the reproductive fitness of flowering plants growing in South Australia. Outcomes will vary dependent upon individual populations and species, geographic location and evolutionary history.



## **Originality statement**

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Phyllis Frances MacGillivray and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Phyllis Frances MacGillivray

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# Chapter 1 Phenological and evolutionary impacts relating to climate change: an overview

## From Philosophy to Science

*“A large number of persons observed extraordinary appearances of the vegetation during the first half of winter 1852-1853 noteworthy to be collected in the annals of meteorology, or even better in the annals of phenology, which is the science of these sorts of things.”* Morren 1852-74, 1853 (Demarée and Rutishauser 2011).

*“There is a sumptuous variety about the New England weather that compels the stranger’s admiration – and regret. The weather is always doing something there; always attending strictly to business; always getting up new designs and trying them on the people to see how they will go.”*

Mark Twain 1835-1910: Speech to New England Society, 22 December 1876.

*“The researches of recent botany have more than once suggested the vague outlines of a truth that, though manifestly requiring investigation, has yet received but little definite expression. It is: - the philosophy that underlies the association of certain groups and types of flowering plants with definite seasons of the year; the principles that will show how this association accords with the “eternal fitness of things.”* (Clarke 1893) p769

*“The most obvious character of the dates of flowering in 1921 is their remarkable earliness’* Francis Darwin writing in the UK 1921, a year when Sir Napier Shaw reported in *The Times* *“The weather of the British Isles ... has been remarkable for its drought, its abundant sunshine and its exceptional warmth”* (Darwin 1921).

Current levels of concern about the long-term impacts of climate change may be a contemporary phenomenon made possible by sophisticated technologies, but such concerns are not unique to our times. In the past, as now, an intimate knowledge of the timing of seasonal events was of great cultural and economic importance (Bolmgren and Lönnberg 2005). The success or failure of annual crops was, and for many still is, a matter of life or death for the people dependent upon them.

In the centuries before scientifically-based weather forecasting, people's livelihoods depended on accumulated knowledge from generations of careful, detailed observations (Sparks, Jeffree et al. 2000; Bolmgren and Lönnberg 2005). Memorable events often came to have cultural and religious significance, such as the flowering of cherry trees in Japan (Frontispiece), where records dating back to the 9th Century (Arakawa 1955; 1956; Aono and Kazui 2008) represent the oldest known phenological series (Koch, Bruns et al. 2008). Careful documentation by natural historians such as Robert Marsham (Marsham 1789) and Henry Thoreau (Dean 2008) has provided many valuable, long-term data sets which now serve as useful biological benchmarks by which to gauge the severity of the current impacts of the rapidly changing global climate (Root, Price et al. 2003; Bardsley 2006; Kullman 2006; Parmesan 2006; Primack and Miller-Rushing 2012).

## Phenology

*“It is not spring until you can plant your foot upon 12 daisies” – a proverb*

Phenology is the study of the timing of seasonal events or, as more poetically described by Bradley, Leopold et al. (1999), “a reading of the pulse of life”. The word itself is a derivation of the ancient Greek *‘phaenesthai’* (φαίνεσθαι), meaning ‘to appear’ (Demarée and Rutishauser 2011), and was first coined by Charles Morren in 1849 to describe “a specific science which has the goal to know the manifestation of life ruled by time” (Demarée and Rutishauser 2011). The status of phenology, as a discipline, has not always reflected its importance (Sparks, Jeffree et al. 2000; Keatley, Fletcher et al. 2002; Chuine,

Yiou et al. 2004), but scientific recognition came with the provision of satisfactory explanations for the coincidental timing of climatic and biological events which had long puzzled and intrigued the early natural historians (Kochmer and Handel 1986; Yanovsky and Kay 2003; Brearley, Proctor et al. 2007). Phenological research has now taken on a new significance and urgency as the need for information escalates. With such potential, its claim for recognition has been acknowledged and its future status secured.

It is now evident that many aspects of the world's physical and biological systems are changing in concert with enhanced climatic warming. Whereas Clarke was able to write with conviction in 1893 (Clarke 1893) that the seasons come and go with prescribed regularity; viz. "spring; late March, April, May: summer; June, July, August: autumn; September and October" (Clarke 1893) p771, rapidly shifting phenologies are now overturning such notions (Zwiers and Hegerl 2008). Clarke was, however, reflecting on events in the temperate conditions of the northern hemisphere where synchronized flowering is a clearly defined and easily observed event (Opler, Frankie et al. 1976; Martínez-Valero, Melgarejo et al. 2001; Meier 2001), heralding the long awaited arrival of spring and marking its onset. In Australia, a more moderate climate allows for year round growing conditions and, unlike the descriptive delineation of seasons in the northern hemisphere, the four seasons in Australia are officially identified with fixed calendar months: Spring (1<sup>st</sup> September- 30<sup>th</sup> November); Summer (1<sup>st</sup> December – 28<sup>th</sup> /29<sup>th</sup> February); Autumn (1<sup>st</sup> March – 31<sup>st</sup> May); Winter (1<sup>st</sup> June – 31<sup>st</sup> August). Thus phrases such as 'the advancement of Spring' have no literal meaning<sup>1</sup>.

## **Transition to flowering**

### ***Phylogenetic***

For plants, phenophases include both the vegetative processes of emergence from underground tubers, bud burst and leaf shedding, and the reproductive processes of bud formation, flowering, and fruit and seed production. The focus of this thesis is restricted to

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<sup>1</sup> It is worthy of note, however, that indigenous Australians base their seasonal calendars on the local sequence of natural events (<http://www.bom.gov.au/lam/ipage.shtml>).

flowering. Noted consistencies within family and lower taxonomic levels indicate that seasonal phenology is to a large extent restricted by phylogeny (Clarke 1893; Kochmer and Handel 1986; Boulter, Kitching et al. 2006). Kochmer and Handel (1986) compared mean flowering data obtained from the Carolinas (USA) with those for the island of Honshu, Japan (north by  $2^0$  of latitude) and found that species of a family often flowered in unison in these widely separated temperate regions. This finding suggests that phenological patterns are intrinsic and relatively stable properties, and upholds the hypothesis of Clarke (1893) who wrote a century earlier “The truth is forced upon us that the various groups of flowering plants are not scattered indiscriminately from one end of the season to the other, but are regulated by definite systematic principles” (Clarke 1893) p770.

### *Seasonal*

Within the constraints imposed by phylogeny and life-form there is a great diversity and complexity of phenological responses (Chinnusamy and Zhu 2009). To maximize their lifetime reproductive success, plants must be able to detect and respond appropriately to the ambient climatic conditions of the region in which they are growing. Anticipation of favourable seasonal conditions, and/or avoidance of unfavourable conditions, requires sensitive responses to changes in day length, light quality, temperature and rainfall (Opler, Frankie et al. 1976; Levy and Dean 1998; Yanovsky and Kay 2003; Peñuelas, Filella et al. 2004; Renner 2007). At higher latitudes, seasonal progression is marked by considerable shifts in day length (Battey 2000) but its relative importance, as the most consistent and reliable cue for seasonal progression, decreases with distance to the equator (Renner 2007).

### *Climatic*

While the initiation of flower buds in some species may depend on quite precise and predictable photoperiodic signals (Fitter, Fitter et al. 1995; House 1997; Law, Mackowski et al. 2000; Renner 2007), this cue is independent of weather and insufficient to signal optimal timing. From a plant’s perspective, temperature detection tends to be a more relevant indicator of seasonal conditions (Fitter, Fitter et al. 1995; Lechowicz 2001), and a



large body of evidence from studies based in the colder, higher latitudes of Europe and America, now categorically links the timing of reproductive events to spring warming (Spano, Cesaraccio et al. 1999; Wielgolaski 1999; Beaubien and Freeland 2000; van Vliet and Schwartz 2002; Parmesan and Yohe 2003; Menzel, Sparks et al. 2006; Clark and Thompson 2010).

At lower latitudes, Mediterranean-type climates present temperatures conducive to growth throughout most of the year (Estiarte, Puig et al. 2011) but are prone to unreliable rainfall such that water becomes the limiting resource and an important constraint (Opler, Frankie et al. 1976; Keatley, Fletcher et al. 2002; Peñuelas, Filella et al. 2004; Mduma, Sinclair et al. 2007; Valtuena, Ortega-Olivencia et al. 2008). Furthermore, plants do not experience temperature or rainfall in isolation, but are affected by overall prevailing weather conditions. Temperature and rainfall interact synergistically, and impact on evapotranspiration, often with very serious consequences in regions where rainfall is unreliable and severe water deficit often coincides with high temperature extremes (Nicholls 2004; Jentsch, Kreyling et al. 2009; Ummenhofer, England et al. 2009).

Droughts can be expected to have particular relevance for plants growing in South Australia, the driest state in the driest continent, with the exception of Antarctica (Law, Mackowski et al. 2000; Simpson and Dean 2002). Drought-tolerant native species have coevolved with the continent's aridification, and successfully adapted to survive the extremes of the Australian climate (Law, Mackowski et al. 2000). Changes in temperature and rainfall patterns are known to translate into shifts in flowering, sometimes to advance, and sometimes to delay, its onset (Law, Mackowski et al. 2000; Keatley, Fletcher et al. 2002; Chambers 2006; Rumpff, Coates et al. 2008). The challenge here is to elucidate and quantify the relationships so predictions can be made in line with ongoing climate change scenarios.

## Climate change

Climate change is expected to have extensive phenological repercussions, both for individual species and the wider ecosystem. There have been two main periods of warming during the last 100 years; the first between 1910 and 1945, and the second from 1976 onwards (IPCC 2007). The rate of warming during the latter period has been approximately double that of the first, and the rate has continued to increase (IPCC 2007). Paleoclimatic data indicates that the rate of warming in the second half of the 20th Century has been the highest for at least the last 1300 years (van Vliet and Schwartz 2002; IPCC 2007).

The Australian climate is undergoing rapid change in line with global tendencies (CSIRO 2008). Average temperatures have risen by approximately  $0.9^{\circ}\text{C}$  since 1910, characterized by increased duration of warm spells and severity and frequency of extreme heat events. Most of this change has occurred since 1950 (Hughes 2003; CSIRO 2008). Best-estimate predictions for 2030 indicate general warming of  $0.7^{\circ}\text{C}$ - $1.2^{\circ}\text{C}$  rising to  $2.2^{\circ}\text{C}$ - $5.0^{\circ}\text{C}$  by 2070, with less warming expected in the south and north-east than the remainder of the continent (CSIRO 2008). A plot illustration of Australia's annual mean temperature anomalies based on a 30-year climatology (1961-1990) is given in Appendix A.1.

Changing weather patterns in South Australia are characterised by reduced cloud cover and rainfall in winter and spring (Hulme and Sheard 1999; Hughes 2003), a decrease in the number of cold days and frosts, and a widespread increase in the incidence of heatwaves (CSIRO 2008). Summer maxima for the Adelaide and Mt Lofty Ranges Region (Bardsley 2006) have increased by up to  $0.85^{\circ}\text{C}$  since 1950, during which time rainfall totals have decreased (Nicholls 2003; 2004). Plot illustrations of South Australia's maximum summer temperature and rainfall anomalies are given in Appendices A.2 and A.3. Future rainfall projections allow for substantial variability and abound with uncertainties, but best estimates of future trends in annual precipitation are for a 10% decrease in southern areas by 2070. This is predicted to coincide with an increase from 17 to 26 days per year with temperatures over  $35^{\circ}\text{C}$  (CSIRO 2008), and is of considerable concern in an already drought prone region.

### **Phenological impacts of climate change**

The intimate relationship which exists between plant phenologies and prevailing climate, coupled with ease of observation, makes phenological monitoring a reliable and cost effective tool for the early detection of change in biological systems (Boulter, Kitching et al. 2006; Cleland, Chuine et al. 2007). To this end, flowering episodes finely tuned to the seasonality of their environment are being analysed world-wide to determine how species are being affected by current global warming (Singh and Kushwaha 2005; Cleland, Chuine et al. 2007; Keatley and Hudson 2007b; Post and Inouye 2008; Primack, Higuchi et al. 2009; Crimmins, Crimmins et al. 2010).

Short-term contemporary studies provide snapshots in time, but are insufficient to determine the magnitude or nature of impacts on large temporal and spatial scales. To place current events in perspective, and to predict future events with confidence, it is necessary to know what has happened in the past (Darbyshire, Webb et al. 2011). Historical records are well placed to provide this reference base (Ahas, Aasa et al. 2002; Keatley, Fletcher et al. 2002; van Vliet and Schwartz 2002; Rosenzweig, Karoly et al. 2008).

Much of the information relating to the phenological impacts of climate change derives from a wealth of historical data which has provided comprehensive scenarios for remote systems of northern Europe and America. They reveal a consistent trend. Since the 1960s, flowering times have become progressively earlier (Fitter, Fitter et al. 1995; Sparks and Carey 1995; Menzel, Sparks et al. 2006; Rosenzweig, Karoly et al. 2008); the advance of early spring-flowering species more pronounced than the later flowering species (Ahas, Aasa et al. 2002; Fitter and Fitter 2002; Cleland, Chuine et al. 2007; Sherry, Zhou et al. 2007). In Britain during the 1990s, an estimated 150 to 200 species flowered on average 15 days earlier than in the very recent past (Root, Price et al. 2003); in Concord, Massachusetts, both native and introduced plant species are currently flowering 10 days earlier, on average, than when first noted by Henry Thoreau in 1850 (Primack and Miller-Rushing 2012). Given the relatively greater rate of warming predicted for the present century (IPCC 2007), future responses are expected to be even more pronounced.

Mediterranean-type systems, such as occur in South Australia, are especially vulnerable (Bardsley 2006) and impacts may already be significant (Chambers, Hughes et al. 2005). However, local knowledge is severely limited in comparison to that of the northern hemisphere, and it is not valid to assume equivalent responses in our southern ecosystems. Baseline data for a broad selection of local flora are urgently needed, and the success of Australian phenological research rests to a large extent on procuring appropriate archival records (Keatley, Fletcher et al. 2002; van Vliet and Schwartz 2002). These can then be interrogated to determine how phenologies have been influenced by the droughts and periods of warming during the last century. Present responses and future impacts can then be gauged against these baseline responses.

*“Historical analysis is a necessary first step when considering the impacts of climate change”* (Darbyshire, Webb et al. 2011).

While there is a relative paucity of long-term phenological records in Australia (Hughes 2003; Rumpff, Coates et al. 2010), they do exist, and include flowering records of plants along the Victorian coast (Gallagher, Hughes et al. 2009); Victorian forest records of eucalypt flowering from 1940 to 1962 (Keatley, Fletcher et al. 2002), and observations of *Pyrus* growing at the Waite Arboretum *Pyrus* in Adelaide (Symon and Gardner 2000; Symon 2002). The most valuable resource archive available to Australian researchers, however, are the collections held in the nation’s herbaria (Borchert 1996; Primack, Imbres et al. 2004; Bolmgren and Lönnberg 2005; Keatley, Hudson et al. 2011; Robbirt, Davy et al. 2011; Panchen, Primack et al. 2012; Proença, Filer et al. 2012). With extensive temporal, geographic and phylogenetic representation, these collections provide the necessary historical foundation to support ongoing research.

Results obtained from such collections have been validated and found to replicate findings from field studies (Borchert 1996; Primack, Imbres et al. 2004), although some discrepancies have been noted by Bolmgren and Lönnberg (2005). While data may not be as complete and precise as records obtained from designed field studies, they nevertheless

reveal characteristic differences between species, and provide valuable clues as to the environmental impact of recent climatic change (Panchen, Primack et al. 2012).

### **Evolutionary implications of climate change**

Throughout evolutionary history, species able to track their bioclimatic envelopes have evaded adverse conditions and been favoured over those which could not (Pearson, Dawson et al. 2002; Beaumont, Hughes et al. 2005). With avenues for dispersal now greatly diminished, and often non-existent, favoured species are those able to survive *in situ* (Root, Price et al. 2003).

Flowering time has a genetic basis, incorporating a level of individual variation as a form of bet-hedging against normal patterns of year to year variability (Levy and Dean 1998; Lechowicz 2001; Simpson and Dean 2002; Weis and Kossler 2004). This phenological plasticity (see page 151) enhances a population's chances of survival well enough in the short term to enable successful evolutionary adaptation in the long term (Fox 1990; Cavers, Navarro et al. 2003; Burke 2004; Franks, Sim et al. 2007; Doi, Takahashi et al. 2010). However, the evolutionary time frames required for advantageous mutations to become fixed in a population may be too vast to replenish depleted populations during this current episode of rapid change (Gingerich 1983; Kochmer and Handel 1986; Lechowicz 2001; Tarayre, Bowman et al. 2007). Hybridization processes may provide a viable alternative.

Natural hybridization is a widespread process with significant evolutionary consequences (Cronn, Small et al. 2003; Culley and Hardiman 2009), and the literature provides many examples of resulting speciation events (Arnold 1997; Neiland and Wilcock 1998; Lopez, Potts et al. 2000; Cronn, Small et al. 2003; Abbott and Lowe 2004). Despite the low success rate of hybrid crosses (Lopez, Potts et al. 2000), their widespread occurrence in nature may be explained by the repeated opportunities which exist for such rare events to occur (Grant 1981). Crossing between closely related species, and between individuals

from well differentiated populations, enriches the gene pool and potentially increases the range of climatic tolerance of the hybrids (Stebbins 1959; Culley and Hardiman 2009). In this way, a changing climate may indirectly lead to the evolution of new hybrid taxa, effectively conserving a species genes, if not the species itself (Potts and Reid 1988; Grant-Downton and Dickinson 2006).

Shifting phenologies may, in turn, impact on the opportunities for hybridization with trade-offs between plasticity and synchrony with interdependent species. Climatically advantageous shifts may compromise timing with specialist pollinators or phenologies of other, closely-related inter-fertile species (de Groot, Ketner et al. 1995; Primack and Miller-Rushing 2012). In other instances, increased synchrony may enhance opportunities for cross-pollination and the establishment of hybrids (Lopez, Potts et al. 2000; Delaporte, Conran et al. 2001; Keatley, Hudson et al. 2004). Whatever the consequences, they can be expected to impact on the wider community network (Beaubien and Freeland 2000; Singh and Kushwaha 2005).

Evolutionary impacts are explored further in Chapter 5.

## **Statistical methods and analysis**

Various methods of statistical analysis have been employed to reveal the trends implicit within phenological data sets. Linear regression has been the major method of analysis used to detect changes in phenological time series (Dose and Menzel 2004; Hudson, Keatley et al. 2005; Sparks and Tryjanowski 2010; Roberts 2011). Traditionally, dates of first (or peak) flowering are plotted against time in years (Bradley, Leopold et al. 1999; Menzel and Fabian 1999; Ahas, Jaagus et al. 2000; Abu-Asab, Peterson et al. 2001; Zheng, Ge et al. 2006; Abu-Taleb, Alawneh et al. 2007; Keatley and Hudson 2012) with the resultant slope of the regression line indicating the mean rate of change of the phenophase given as days per year. *Major* limitations of this least squares approach are its unreliable extrapolation properties, restriction to time-series with a comparatively linear trend, and sensitivity to outliers and boundary values. Only rarely have other curve fitting

methods been used to detect change (Ahas 1999; Sagarin and Micheli 2001; Roberts 2008; Hudson, Kim et al. 2010).

Throughout this thesis, regression methods have been used for initial exploratory analyses (James and McCulloch 1990), and where practicable, followed up with more sophisticated analytic methods better suited to the exploration of large, complex, herbarium-based data sets. Generalized Additive Models for Location, Scale and Shape (GAMLSS) (Rigby and Stasinopoulos 2001; Stasinopoulos and Rigby 2007) were chosen as the most appropriate curve-fitting modelling tool on the basis of their ability to allowing for non-linear impacts of the explanatory variables (Hudson, Kim et al. 2010).

### **Thesis research outline**

This thesis has, as its central concern, the impacts of climate change - past, present and future - on flowering phenology in South Australia. Other phenophases, although important, are not considered. Past phenological and meteorological records are interrogated using a variety of statistical methods to ascertain the main climatic drivers of shifts in flowering phenology. On the basis of these findings, predictions are made of the likely consequences for species' fitness, and the future health and functioning of regional ecosystems.

My research has focused on the phenology of two very different genera: the locally endemic *Diuris* orchids and the introduced *Pyrus* (pears). These particular taxa were chosen as they were each represented by quality, long-term data sets which allowed for comparison between two different life forms, and the responses of well adapted, and non-adapted, species. Additionally, the orchids satisfy many of the criteria required of bioindicator species, while the pears, better adapted to a cooler and wetter climatic zone, enable comparisons to be made with their northern counterparts. They also have economic implications for the local horticultural industry. Proximity of field sites also favoured supporting and ongoing field observations.

The following chapter (Chapter 2) documents an initial interrogation of herbarium records of three representative *Diuris* species to establish the temporal nature of phenological shifts over their entire historical range. This investigation is extended in Chapter 3 to determine probable climatic drivers of these shifts in line with contemporary meteorological data. In Chapter 4 the focus and location shifts from native herbaceous species to the non-native horticultural pears growing at the Waite Arboretum. This observational data set offered the additional opportunities to assess differences between individual trees, and compare their southern phenology with that from the northern latitudes of their evolutionary origin.

The possible evolutionary impacts of ongoing climate change on these species, and the potential use of phenological bioindicators as a practical means for monitoring species fitness and ecosystem functioning are addressed in Chapters 5 & 6. This PhD thesis concludes with a summary of the changing nature of phenological studies and their contribution to management and conservation.



## **Chapter 2 Herbarium collections and photographic images**

Published as Chapter 19 in *Phenological Research: methods for environmental and climate change analysis* (Keatley and Hudson 2010)

### **19 Herbarium collections and photographic images: Alternative data sources for phenological research**

Fran MacGillivray<sup>1,4</sup>, Irene Hudson<sup>2,3</sup> and Andrew J Lowe<sup>1</sup>

<sup>1</sup>*Australian Centre for Evolutionary Biology and Biodiversity, School of Earth and Environmental Sciences, The University of Adelaide, Adelaide, South Australia*

<sup>2</sup>*School of Mathematics and Statistics, University of South Australia, Adelaide, South Australia*

<sup>3</sup>*Institute for Sustainable Systems and Technologies, University of South Australia, Mawson Lakes, South Australia*

<sup>4</sup>*Correspondence author: Room 205a Darling Building, University of Adelaide, North Terrace, Adelaide, 5005 South Australia. Email: phyllis.macgillivray@adelaide.edu.au.*



## Statement of Authorship

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Name of Principal Author (Candidate)	Phyllis (Fran) MacGillivray		
Contribution to the Paper	Sought and obtained funding; performed initial analysis on all data; interpreted all analyses; wrote manuscript as principal author with contribution from Irene Hudson*; acted as corresponding author		
Signature		Date	26 Oct 2012

Name of Co-Author	Irene L Hudson		
Contribution to the Paper	Performed all GAMLSS analysis; contributed to section of manuscript relating to GAMLSS methodology; helped to evaluate and edit the manuscript		
Signature		Date	19 October 2012

Name of Co-Author	Andrew J Lowe		
Contribution to the Paper	As principal supervisor, advised on development of the manuscript.		
Signature		Date	23.10.12



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## Introduction

In the past, as now, an intimate knowledge of the timing of seasonal events was of great cultural and economic importance (Bolmgren and Lönnberg 2005). Before the advent of scientifically based weather forecasting, people's survival and wellbeing depended to a large extent on accumulated knowledge from generations of careful, detailed observations (Sparks, Jeffree et al. 2000; Bolmgren and Lönnberg 2005). Memorable events sometimes came to have cultural and religious significance, such as the flowering of cherry trees in Japan for which records from Kyoto date back to the 9th century (Arakawa 1955; 1956) and constitute the oldest known phenological series (Koch, Bruns et al. 2008). Dedicated amateur natural historians such as the Marsham family in the U.K. from 1736 to 1947, Henry Thoreau from 1837 to 1861 and, more recently, Mrs. Anderson from 1960 to 2002 in Massachusetts USA (Dean 2000; Ledneva, Miller-Rushing et al. 2004; Miller-Rushing and Primack 2008) have provided a further legacy of valuable long-term data sets which are now providing evidence that many aspects of the world's biological systems are changing in concert with current enhanced global warming. Consequently, phenological research has taken on a new significance and urgency. Answers are being sought to fundamental questions about the implications of climate change for species survival and ecosystem function in which phenology is now recognized as a driver of dynamic ecological processes as opposed to merely a passive response to environmental change (Primack, Miller-Rushing et al. 2007; Post and Inouye 2008).

In most cases however, long-term historical observations of phenology are the exception rather than the rule, and information needs to be acquired from alternative data sources. This chapter is specifically focused on accessing long-term phenological data from specimens residing in herbarium collections and photographic images. We outline the constraints in linking phenological changes with climatic fluctuations and long-term trends, make some specific observations about herbaria and photographic data, offer some cautionary principles for analysis and interpretation and finally offer two case studies where phenological data have been successfully extracted from herbarium records. In contrast to northern hemisphere regions, where long-term biological data sets are frequent and diverse (Europe, USA, Japan), southern hemisphere records are an order of magnitude less frequent. The potential to access phenological information from alternative data

sources such as herbaria and photographs therefore represents a very useful resource for South America and Africa, and particularly for Australia where more than six million herbarium specimens exist. <http://www.anbg.gov.au/chah/avh/avh.html>

### **Constraints to linking phenology with climate**

Phenology encompasses a wide range of vegetative and reproductive responses to local environmental conditions. However, consistencies have long been observed which indicate that phenological patterns are intrinsic and relatively stable properties of taxon groups (Clarke 1893; Kochmer and Handel 1986; Boulter, Kitching et al. 2006). Phylogenetic constraints have been defined as those ‘properties shared by members of a monophyletic group by virtue of their common ancestry which limit the response of these taxa to directional selection’ (Kochmer and Handel 1986). One of the first to suggest and document this, in relation to flowering time, was Clarke (1893) who wrote more than a century ago “*The truth is forced upon us that the various groups of flowering plants are not scattered indiscriminately from one end of the season to the other, but are regulated by definite systematic principles*” (Clarke 1893) p 770, and it has indeed proven to be the case. However, within the constraints imposed by past evolutionary adaptations and shared through lineage relationships, there is a great diversity and complexity of phenological responses (Colasanti and Coneva 2009).

The synchronization of a plant’s vegetative and reproductive biology with local environmental conditions is essential for maximization of its chance of reproductive success. To this end, plants need to detect and respond to environmental cues that indicate incipient seasonal changes, both favorable and hostile to their ongoing development. The most reliable indicator of seasonal progression is photoperiod. As seasonality and changing day length are dependent upon distance from the equator, photoperiod monitoring is imperative for daylength dependent transition to flowering at higher latitudes (Simpson and Dean 2002). Plant species have evolved distinct reproductive strategies by which they can respond very precisely to relatively small changes in the ratio of daylight to darkness (Rivera and Borchert 2001; Simpson and Dean 2002; Yanovsky and Kay 2003). However, despite its fundamental importance, the influence of



photoperiod is independent of climate and environmental conditions, and alone is insufficient to determine the procession of plant phenological responses.

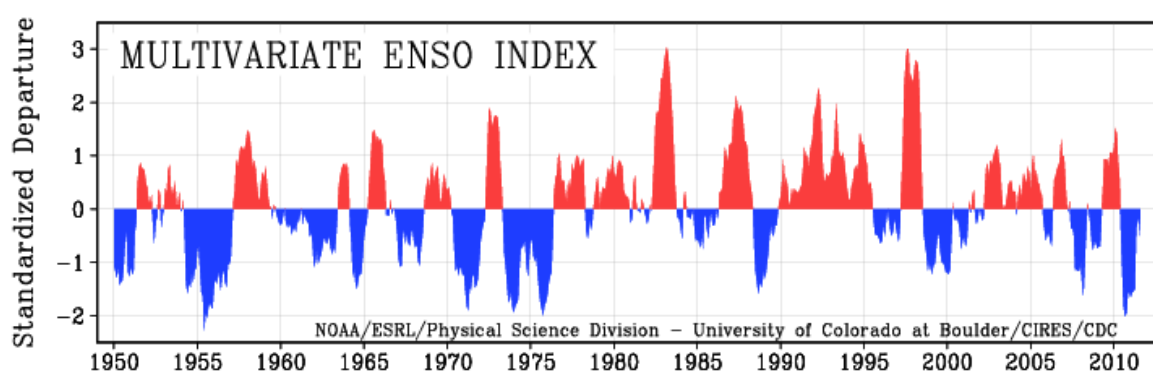
## **Weather and climate**

Superimposed on the seasonal regularity of photoperiodism are more highly variable and generally less predictable climatic factors such as temperature and precipitation (Spano, Cesaraccio et al. 1999; Lechowicz 2001; Brearley, Proctor et al. 2007; IPCC 2007). Temperature has a major influence on plant developmental responses. Accumulated temperature above a certain threshold value, usually expressed as degree-days from the first of January, is a major trigger for the onset of flowering (Spano, Cesaraccio et al. 1999; Beaubien and Freeland 2000), and accumulating evidence from the northern hemisphere links the timing of phenological events to the onset of spring warming (Spano, Cesaraccio et al. 1999; Wielgolaski 1999; Beaubien and Freeland 2000; van Vliet and Schwartz 2002; Walther, Post et al. 2002). Temperate and cold zones are particularly sensitive to changes in climate (Ahas, Aasa et al. 2002; Sparks and Menzel 2002; Rutishauser, Luterbacher et al. 2007) and shifts towards the earlier onset of spring phenophases have been more pronounced in these regions than nearer the equator (Parmesan 2007; Rutishauser, Luterbacher et al. 2007).

Global patterns of change have been attributed to changes in large-scale climatic processes such as the North Atlantic Oscillation (NAO) (Scheifinger, Menzel et al. 2002), the Arctic Oscillation (AO) (Min, Zhang et al. 2008) and the El Niño Southern Oscillation (ENSO) (Wolter and Timlin 1993; 1998; Beaubien and Freeland 2000; Schaubert, Kelly et al. 2002; Walther, Post et al. 2002; Brearley, Proctor et al. 2007) (Fig. 1). There were two main periods of warming during the 20<sup>th</sup> century; the first between 1910 and 1945, and the second from 1976, marking the beginning of an extended warm El Niño phase (Wolter and Timlin 1993; 1998; IPCC 2007) (Fig. 1). Records from the Australian Bureau of Meteorology show that the period since 1980 includes many of the warmest years on record for Australia, with considerable increases in both annual maximum and annual minimum temperatures <http://www.bom.gov.au/climate/change/amtemp.shtml>. The rate of

warming during this period has been approximately double that of earlier in the century, and the rate has continued to increase (IPCC 2007).

Paleoclimatic data indicates that the rate of warming since 1950 has been the highest for at least the last 1300 years (van Vliet and Schwartz 2002; IPCC 2007). Estimates of projected future climate change (IPCC 2007) indicate that, by 2030, rates of global warming could very likely be at least double that observed for the 20th century, manifesting as more frequent and extreme heat waves, and heavy precipitation events brought on by the increasing frequency and intensity of El Niño events (Walther, Post et al. 2002) as have occurred after 1976 (Wolter and Timlin 1993; 1998) (Fig. 1).



**Fig. 1** Multivariate ENSO Index. Positive MEI values indicate the warm El Niño phase; negative values the cold La Niña phase (MEI). The El Niño events of 1982-83 and 1997-98 which rank as the highest of the century (Wolter and Timlin 1998) fall within the period (1976-1998) during which there is a noticeable reduction of La Niña events. Sourced from <http://www.cdc.noaa.gov/people/klaus.wolter/MEI/> (5/5/2009).

Regional precipitation is more erratic and less dependable than temperature, with years of good rainfall interspersed with sometimes extended periods of drought. Various aspects of both temperature and precipitation need to be considered both separately and in combination to unravel the synergistic interactions among these parameters (Keatley and Hudson 2007a), and careful analysis required to tease out signals from within this complex system. Temperature and rainfall interact to impact on processes such as evapotranspiration with very serious consequences in regions where rainfall is unreliable; and severe water deficit tends to coincide with high temperature extremes. These

interactions will have particular impact in areas such as southern Australia, where a year round growing season and high maximum temperatures often coincide with periods of low precipitation and drought, both of which are predicted to increase in frequency and intensity (IPCC 2007; Chambers 2008; CSIRO 2008) and must be given overriding consideration.

Broad-based multi-taxon analyses have indicated a mean response to recent warming with advances of spring events, estimated at between 2.3 and 2.8 days/decade (Parmesan and Yohe 2003; Root, Price et al. 2003; Parmesan 2007), reflecting the current enhanced warming trend. With regard to the higher northern latitudes where cold winters, often augmented by snow and ice cover, restrict the growing season (Rutishauser, Luterbacher et al. 2007), research focus has been on minimum temperatures. While plants do not experience temperature or any other aspect of weather in isolation, other climatic parameters such as rainfall and periods of drought, while influential, are not considered to be major drivers of phenology in these northern regions (Wielgolaski 1999; Rutishauser, Luterbacher et al. 2007). However, they do take on much greater relative importance for rainfall limited ecosystems, such as occur in Africa and Australia (Johnson 1992; Dreyer, Esler et al. 2006; CSIRO 2008).

Opportunely, Australia's endemic plant species have had the benefit of millennia to evolve effective responses to the climatic extremes and irregularities typical of the continental weather systems. A good example can be seen in the reproductive ecology of some species of *Eucalyptus*. Unencumbered by an urgency imposed by a restricted growing season, these trees have adapted instead to cope with a continuous growing season plagued by uncertainty. Thus, while initiation of flower buds in some species may be in response to quite precise and predictable photoperiodic signals (Fitter, Fitter et al. 1995; House 1997; Law, Mackowski et al. 2000; Renner 2007), the onset of flowering can be withheld, sometimes for many months, until conditions become suitable (Fitter, Fitter et al. 1995; Lechowicz 2001; Keatley, Fletcher et al. 2002). Keatley, Fletcher et al.(2002) found that the combined effect of temperature and rainfall had more explanatory power in relation to the flowering commencement of four eucalypts species than either factor showed when considered separately.

By comparison, the species *E. regnans*, when growing at elevations above 600 meters, with adequate water supply, exhibits a more stable reproductive cycle with relatively specific budding and flowering periods influenced predominantly by summer and autumn temperatures, and photoperiod (Ashton 1975). Flower buds, first detected in late spring to late summer, swell during the following November and December and finally come into full flower more than two years later during the shortening days of autumn when three generations of buds may be present on the same branch. Flowering tends to be earlier at lower altitudes and on younger trees, but considerable variation is found among individual plants. A considerable variability in flowering abundance, which conforms to a more or less regular two or four-yearly pattern may, however, be disrupted during periods of drought.

Generally, however, satisfactory explanations for the coincidental timing of climatic and biological events have been difficult to ascertain, and details of the mechanisms regulating ecosystem processes in general remain unknown. At the same time, our dependence upon the consequences of such processes has become an issue of utmost importance. Throughout the world native plants may be well adapted to the vagaries of their regional climates, but they now must face increased challenges while coping with adversity imposed by habitat loss and destruction, the introduction of pests, and other human related activities. Thus, after millennia of remarkable and successful adaptations, the future of many endemic species is now uncertain.

### **Using phenology to assess complex relationships between species and climate through time**

Irrefutable evidence is emerging from the scientific literature of universal shifts in phenology as a consequence of climate change, now widely expected to have a number of major impacts on biological systems around the globe. Mediterranean systems such as occur in southern Australia are especially vulnerable (Bardsley 2006). The intimate relationship which exists between seasonal flowering and climatic conditions, coupled with ease of observation, makes phenological monitoring of flowering events a reliable and cost effective method for the early detection of change in biological systems and an

important tool in global change research (Keatley, Fletcher et al. 2002; Menzel 2002; Keatley and Hudson 2007b; Keatley, Chambers et al. 2008; Keatley and Hudson 2008).

Even when rapid, climate change is a relatively long-term process and observations on decadal timescales are insufficient to place phenological shifts in perspective. Short-term phenological studies provide important snapshots in time, but cannot be used to determine the magnitude or nature of impacts on the large temporal and spatial scales of the processes themselves. Much of what we know about the influence of climate on phenology comes from investigations of archival records undertaken in the northern hemisphere. For these we are indebted to the dedication of amateur naturalists (Marshall 1789; Dean 2000; Dean 2008; Post and Inouye 2008), many of whose observations and collections were made at a time when environmental concern was directed at the recently arrived railroads and their trains steaming across their landscapes (Fender 1997), proclaiming the industrial revolution and enhanced input of greenhouse gases into the atmosphere.

### **Historical data sets**

While not always conforming to modern, systematic standards and often highly subjective, many historical recordings made by these motivated, knowledgeable and extremely precise observers, now provide us with a valuable source of reliable phenological information (Rutishauser, Luterbacher et al. 2007; Dean 2008; Miller-Rushing and Primack 2008). Although such records are by nature variable, discontinuous, and incomplete, they have been shown to furnish comparable results to those obtained from field investigations (Primack et al. 2004, Miller-Rushing et al. 2006) and provide supporting evidence that flowering times are indeed responding to current warming trends. Some species in Massachusetts are now flowering seven days earlier than they did 150 years ago in Thoreau's day (Dean 2000; Miller-Rushing, Primack et al. 2006; Dean 2008).

In many countries, particularly those of the southern hemisphere, equivalent long-term data equivalents are scarce: Marshall was making the first of his recordings more than 50 years before the first fleet sailed out of Portsmouth bound for Botany Bay, Australia. As it is inappropriate to extrapolate from findings derived from northern hemisphere data sets, however sound they may be, researchers in the southern hemisphere must turn to

alternative, local sources for urgently needed information on a comprehensive selection of plant species. Collections held in national herbaria satisfy these criteria, and the immediate future of phenological studies in the southern hemisphere, particularly Australia, rests to a large extent on this resource.

### ***Herbarium collections***

The application of herbarium vouchered specimens to comparative phenological studies is a relatively recent approach, but initial explorations have produced convincing evidence of their suitability and led the way to their wider application (Borchert 1996; Primack, Imbres et al. 2004; Bolmgren and Lönnberg 2005; Lavoie and Lachance 2006; Gallagher, Hughes et al. 2009) (Table 1).

Collections comprise a broad temporal, geographic and phylogenetic representation of their regional flora which are conveniently available independent of season. In particular, they have been found to provide a reliable estimate of the peak flowering date, a convenient measure of flowering time as it is relatively independent of population size (Primack and Miller-Rushing 2009). Indeed vouchered specimens, used either alone or in combination with field observations, can show that flowering times respond to changes in temperature and reveal intraspecific variations in flowering phenology relative to seasonal conditions throughout a species' entire range (Borchert 1996). They thereby offer a reliable data source (Primack, Imbres et al. 2004; Bolmgren and Lönnberg 2005) with the potential to provide valuable supporting evidence of phenological changes in response to global warming (Miller-Rushing, Primack et al. 2006; Sparks 2007).

## Herbarium collections

**Table 1.** An overview of studies investigating the suitability of herbarium collections for the detection of climate-change impacts on flowering phenology.

Reference	Data source	Investigation	Main measure: Analysis	Region	Taxa investigated
Borchert 1996	Missouri Botanical Garden herbarium collections	Comparison of flowering information obtained from collections and field data	Month of flowering: Graphical comparisons	Central and South America N.H./S.H.	18 Neotropical dry forest species
Rivera and Borchert 2001	Herbario Nacional, San José, Costa Rica, Missouri Botanical Garden, St. Louis, Mo.	Inter-annual variation in flowering periodicity	Flowering period: Graphic representation of flowering period and other phenophases	Costa Rica Central Americas N.H./S.H.	> 25 tree species; herbs
Bolmgren and Lönnberg 2005	Swedish Natural History Museum	Evaluation: herbarium flowering-time data	Collection dates: Product-moment correlations Wilcoxon matched-pairs test	Uppland, Sweden N.H.	77 species
Lavoie and Lachance 2006	Canadian herbaria	Phenology reconstruction	Flowering dates: Linear regression	Quebec, Canada N.H.	Tussilago farfara
Buggs 2007	Review of herbarium data	Historical evidence of hybrid zone movement		N.H./S.H.	Multi-taxon
Rumpff et al. 2008	3 Australian herbaria	Determination of indicator species	First flowering date: Linear regression	Victoria Australia S.H.	79 coastal species.
Lavoie and Lachance 2006	Missouri Botanical Garden's TROPICOS database	Predicting species distributions	Presence of flowers: maximum entropy models	Bolivia, Ecuador S.H.	323,711 voucher specimens of 76 species
Gallagher et al. 2009	3 Australian herbaria electronic data	Phenological trends	Flowering dates: Linear regression	Australian alpine zone S.H.	7 families, 20 species

N.H. = northern hemisphere, S.H. = southern hemisphere

In Australia, herbaria collectively house in excess of six million vouchered specimens dating from the earliest days of European settlement; an historical record of over two hundred years. By its nature, a large component of this data source is prone to error, especially in relation to spatial positioning and scientific naming, and the process of digitization has incorporated careful revision. While a very time consuming, and therefore expensive process, the end product with errors minimized, is highly reliable, comprehensive and readily accessible.

Australia's Virtual Herbarium (AVH), a collaborative project of Australian State, Territory and Commonwealth herbaria, delivers its product via an electronic Australian Flora on-line with data storage and retrieval via the world wide web: <http://www.anbg.gov.au/chah/avh/avh.html>. It provides a wealth of botanical data associated with scientific plant specimens held in these major Australian herbaria (Fig. 2), many enhanced by images, descriptive text and identification tools. It has the advantage of including records from multiple sources without the duplication which arises when the same data is obtained independently from each of the contributing institutions. A global equivalent, the Index Herbariorum, comprises a detailed directory of the public herbaria of the world. This joint project of the International Association for Plant Taxonomy and the New York Botanical Garden lists above 3300 herbaria and includes a photographic archive and is available at <http://sciweb.nybg.org/science2/IndexHerbariorum.asp> (Holmgren and Holmgren 1998 ).





**Fig. 2** Photograph of vouchered specimen *Diuris pardina* BS128-3730, record number AD176137, held at the State Herbarium of South Australia, Plant Biodiversity Centre, Adelaide. See Table 2 for the information provided by Australia's Virtual Herbarium (AVH) for this specimen.

**Table 2.** Information relating to vouchered specimen of *D. pardina* BS128-3730, #AD 176137, as obtained from the AVH data base. See Figure 2 for photograph of the actual specimen held at the State Herbarium of South Australia. N.B. Collection date as formatted does not specify century of collection. This particular specimen was collected in 2004.

Family	Angiosp.
Genus	<i>Diuris</i>
Species	<i>pardina</i>
Scientific Name	<i>Diuris pardina</i> Lindl.
Herbarium Source	AD
Record Suffix	176137
Latitude	-34.54861
Longitude	135.71833
Nearest named place	Wanilla
State	South Australia
Country	Australia
Collection Date	3-Sep-04
Collector	Lang, P.J.
Collecting number	BS128-3730
Additional collectors	
Geocode Precision	150
Coordinate Error Method	GPS
Date Last Edited	11-Dec-06

However, the archival record available to us today has not arisen from an interest in, or concern about, climate change. Its value in this respect has only become evident in retrospect, and analysts investigating these collections, made by someone else for purposes other than those being analyzed (Miller-Rushing and Primack 2008), must deal with the restrictions this imposes upon them. Those who work directly from original sources find that old records do not always give up their secrets easily, and as with all valuable collections, care needs to be taken. Until relatively recently, field naturalists did not have the convenience of the world-wide-web or word processors to keep them updated on the latest taxonomic developments or to redress their messy handwriting and, without the strict guidelines imposed on the collectors of today, many otherwise valuable early records lack collection date and/or location details and therefore cannot be considered in this type of scientific analysis (see case study “Tracking phenological shifts and evolutionary impacts relating to climate change” page 40). Leopold and Jones (1947)

demonstrate the benefits to be derived from the careful and systematic organization of data, a practice not made obsolete by the advent of the computer. While they did not consider the impacts of climate change in their report covering the decade 1935-1945, they discuss many pertinent issues and possible sources of error including those arising from multiple observers and differing intensity of observations.

While dated vouchered specimens, collectively, may provide a good indication of peak flowering date, each individual specimen can provide only an approximation of that event. This is especially the case for plants with long flowering periods, when any one specimen could be collected several weeks earlier or later than the actual occurrence (Primack and Miller-Rushing 2009). Relatively large sample sizes are needed for this assumption to be valid. When compared with field data, flowering times derived from herbarium specimens also tend to be later for early spring-flowering species, and earlier for late summer-flowering species (Bolmgren and Lönnberg 2005). This may be explained by the fact that early-flowering species are typically collected later, and late-flowering species, earlier, in their relative flowering periods. The length of a species flowering period gauged from herbarium collections is usually greater than that obtained from geographically restricted and time limited field investigations but shorter than that suggested by published floras (Bolmgren, Eriksson et al. 2003; Bolmgren and Lönnberg 2005).

### *Collection effort*

It is well recognised that collection efforts are not consistent over time, but may vary from species to species, location to location, and from year to year. Early records are often sparse, as are those coincident with adverse periods in history, such as the two world wars. Short-term anomalous increases may correspond to periods in which field investigators are employed or field naturalists with a special interest make a particular endeavour. Resulting bias may lead to a false interpretation of flowering time variation, reflecting collection effort rather than climate change. Concerns have also arisen in relation to possible roadside and other collection bias associated with geographic distribution and the different climatic conditions associated with sampling locations (Rumpff, Coates et al. 2008).

Loiselle, Jørgensen et al.(2008) found that although climatic gradients were non-uniformly and poorly represented in herbarium collections, this did not detract from the overall value of the data set (Loiselle, Jørgensen et al. 2008).Where necessary, correction procedures should be applied to account for these parameters when using herbarium specimens to reconstruct flowering dates (Kochmer and Handel 1986; Johnson 1992). Although elevation is now required for each vouchered specimen submitted to a herbarium, the AVH database does not provide information specifying elevation of collection location, and this must to obtained by other means if necessary. Spatial coordinates are generally, but not always, provided.

With such irregularities as these making it difficult to extract a clear signal from the data, recourse to expert opinion is always advisable (Walther, Post et al. 2002; Rumpff, Coates et al. 2008). However, when allowances have been made and precautions taken, these data sets have the potential to make an outstanding contribution to studies in comparative phenology. Especially now, with many of the early specimens representing former distributions, species on the verge of extinction, or already extinct, these collections are beginning to take on a special significance. Recent searches for the plants recorded by Thoreau highlight the plight of many; of the 21 species of orchids Thoreau observed in Concord, only seven are known to exist in the area today (Dean 2008). Paradoxically, with herbarium records now accessible as never before, collection efforts appear to be on the decline (Prather et al. 2004, Watanabe et al. 2006). Alternative sources of information, however valuable, cannot replace the need for the actual specimens, the “fundamental records of biodiversity” (Prather, Alvarez-Fuentes et al. 2004) p 216, which will continue to provide the crucial infrastructure and reference base upon which phenological and other floristic research depends. They may, however, fulfil other roles and provide opportunities for phenology studies to proceed in a new, and hitherto inaccessible, direction.

### *Completing the picture*

While we cannot return to the past to ‘fill in’ the inevitable gaps in the record, statistical methods for the analyses of time series with missing values have been developed to compensate for this deficiency (Fornaciari, Orlandi et al. 2003; Golyandina and Osipov

2007). Furthermore, researchers are now exploring the potential of photographic images to supplement herbarium collections. Flowering phenology has always had popular appeal and attracted the attention of many photographers who, unsuspectingly, have bequeathed a rich resource of pictorial knowledge. These are less likely than specimens to have found their way into the major archives, and many undoubtedly still await discovery in wooden filing cabinets or on dusty shelves in local museums and private collections. Initial investigations have demonstrated the potential of dated photographs with referenced locations to reliably augment other sources used to assess shifts in flowering times (Sparks, Huber et al. 2006; Sparks 2007) (Table 3). They have been found to provide robust estimates comparable with those derived from field investigations and broadly in keeping with those published in the literature. As for herbarium specimens, assumptions of peak flowering have been validated, providing confidence in the evidence of change obtained from this metric, both in relation to time and to differences in past and present temperatures (Bolmgren and Lönnberg 2005; Miller-Rushing, Primack et al. 2006; Sparks 2007). Visual records may also provide information on plant size, a confounding factor in flowering phenology (Primack and Miller-Rushing 2009).

Additional information can be retrieved from photographic time series obtained by repeat or time-lapse photography at permanent monitoring sites; a process indispensable for capturing changes over time (Watanabe, Ohokubo et al. 2006). Historical photo-point time-series, however, tend to be in black and white and are often restricted to an annual series which more generally captures a landscape perspective. They are, perhaps, more appropriate for the recording of phenophases other than flowering, such as leaf flushing or the emergence of winter grasses, along with indications of periods of drought and other seasonal and climatic changes (Sinclair 2004; 2005).

**Table 3.** An overview of studies investigating the suitability of photographic images for the detection of climate-change impacts on flowering phenology.

Reference	Data	Investigation	Main measure:		Taxa Investigated
			Analysis	Region	
Photography (archival)					
Sinclair 2004	fixed date, fixed subject photographs	landscape changes following exclusion of grazers	descriptive	Koonamore South Australia S.H.	Arid zone vegetation including <i>Acacia</i> , <i>Casuarina</i> , <i>Myoporum</i> spp
Sparks et al. 2006	fixed-date, fixed subject photographs: Weather-wise (Willis 1944)	influence of weather variables	plant development scores: ANOVA, Multiple regression	United Kingdom N.H.	<i>Galanthus</i> , <i>Narcissus</i> , <i>Aesculus</i> , <i>Fagus</i> spp.
Sparks 2007	photographs	Review			
Primack et al. 2007	archival collections: 1) Arnold Arboretum 2) Landscape photographer	Effects of climate change on flowering times	Flowering dates: Linear regression	1) Boston Mass. USA 2) Concord Mass. USA N.H.	1) Various cultivated woody plants 2) Various wildflowers, trees and shrubs
Photography (digital)					
Crimmins and Crimmins 2008	repeat photography: Nikon D70 digital SLR camera	Phenological monitoring	Estimate of greenness; individual flower counts: Image analysis; mathematical algorithms	Tucson, AZ. USA N.H.	Native wildflowers
Ahrends et al. 2008	standard digital 5-megapixel NIKON Coolpix 5400, CCD sensor	leaf unfolding	date of leaf emergence: image analysis based on RGB colour	Switzerland N.H.	Mixed beech forest including <i>Fraxinus</i> , <i>Fagus</i> spp.

N.H. = northern hemisphere, S.H. = southern hemisphere

### *Digital photography*

But it is not only climate which is changing; advancing technology is also making an impact on our world and the methods by which we interpret it. A growing catalogue of digital images, accompanied by a wealth of pertinent and readily shared information (Fig. 3, Table 4), is dramatically expanding the available phenological data base. Until now, the accurate capture of a key phenological event has necessitated regular, labour intensive monitoring, beginning some days prior to its expected occurrence (Rivera and Borchert 2001). Automatic digital repeat or time-lapse photography, freed from the restrictions imposed by a grey colour scale, the need for photographic film and constant human intervention, is ideally suited to these demands (Crimmins and Crimmins 2008). Resulting images reliably capture not only the actual phenological events, but also quantitative changes over time, such as shifts in the abundance of flower blooms, their pollinators and herbivores (Crimmins and Crimmins 2008; Faast and Facelli 2009).



**Fig. 3** Digital photograph of yellow form of *Diuris orientis* (Orchidaceae). Photographer Jennifer Skinner.

**Table 4.** Digitally recorded information relating to digital photograph (Fig. 3) of yellow form of *Diuris orientis* (Orchidaceae).

Date and Time	22/09/2008 ; 9.20am
Location	Belair National Park, South Australia.
Camera	Pentax K10D
Image ID	IMGP7783.JPG
Image size	3.96MB
Settings	ISO 100, f 32, exposure time 1/90, 80mm focal length

More recent technological initiatives have led to the coupling of cameras with edaphic and meteorologic recording devices. Such integrated networks are capable of augmenting digital images with sufficient site-specific information to enable a precise determination of environmental triggers of the events observed (Sparks, Huber et al. 2006; Ahrends, Brügger et al. 2008; Crimmins and Crimmins 2008). However, systems are still under trial, and reports to date have been limited to experimental plots. Conversion for larger scale field investigations will require several important modifications, including weatherproof housing for camera and computer equipment, an external power source (electricity, battery or solar panel) and, in some locations, insulation from temperature extremes. However, they promise unprecedented opportunities to obtain the wealth of data needed to answer the wider questions about the implications of global climate change in relation to ecological processes and ecosystem function. To this end they can be archived as a permanent record readily available to future researchers.

### **Cautionary points for analysis and interpretation**

As always, the value of such archival data sets as a source of phenological information depends to a very large extent on the methods of analysis employed and the interpretation of the results obtained. Some points for consideration include:



## Herbarium collections

- the non-independence of data due to phylogenetic constraints, multiple collections on any one day at any one site
- the validity of assumptions that sampling has been random with respect to geography, climatic and environmental gradients, etc.
- natural variation across sites, including local microclimatic conditions, genetic differences and other non-climatic factors contributing to uncertainty
- geographical limits (latitude, longitude, elevation)
- bias resulting from inconsistent collection effort
- environmental changes throughout the period of collection such as deterioration and loss of habitat, conservation status of species, etc.
- increasing impact of urban heat island effects confounding climatic warming signals
- appropriate flowering time measure for the data set under consideration (date of first flowering, date of peak flowering, length of flowering time, changes in flowering abundance, etc.)
- non-linearities inherent in the time series
- non-uniform periods of warming which have occurred throughout the 20th century and the particular challenge they pose when linear regression analysis is used for the reconstruction of trends
- error may be introduced through taxonomic issues, nomenclatural inconsistencies, inaccurate recording of spatial coordinates, etc.
- detection of signals indicative of earlier flowering may need to be very strong to be discerned above noise in the data
- ways of improving the accuracy and reducing the uncertainty in analyses, and the limitations, assumptions and uncertainty impose on the interpretation of the results.

## Two Australian case studies

### *Case Study 1: Phenological trends among Australian alpine species: using herbarium records to identify climate-change indicators*

Until recently, the major investigations into flowering phenology of plants in southern Australia have been obtained from State Forest Commission records of *Eucalyptus* species (Keatley, Fletcher et al. 2002; Keatley, Hudson et al. 2004; Barbour, Potts et al. 2006; Keatley and Hudson 2007a). The recent study by Gallagher, Hughes et al. (2009) represents a departure from this precedent with a multi-taxon study exploring the strengths and limitations of herbarium voucher specimens for the detection of changes in flowering phenology of Australian alpine plants in the vicinity of Mt. Kosciuszko. This region has a unique climate for the Australian mainland, but is comparable to many regions in the northern hemisphere where an intimate link between the timing of snowmelt and flowering plant phenology is well established (Lavoie and Lachance 2006; Rutishauser, Luterbacher et al. 2007; Inouye 2008). Temperature sensitivity in relation to flowering time in these alpine regions is likely to be more pronounced than elsewhere on the Australian continent, and rising temperatures, accompanied by a 40% reduction in snow cover since the 1960s, have special significance (Green and Pickering 2002; Pickering, Good et al. 2004; Gallagher, Hughes et al. 2009).

This study entailed a search for suitable indicator species and potential locations for proposed monitoring sites. A preliminary list of 171 potential candidates representing the flora of the alpine region and the Victorian Alps at elevations above 1500m was selected from Kosciuszko Alpine Flora (Costin, Gray et al. 2000). Data for these species were then obtained from three of Australia's major herbaria: the National Herbarium of NSW, the National Herbarium of Victoria and the Australian National Herbarium in Canberra. Duplicate records were removed from the combined data sets, as were:

- records with insufficient, or incorrect, collection date or location information
- multiple observations of a species in any one year (the first observation only was retained)

## Herbarium collections

- all observations not falling within the period 1950-2007 inclusive (to correspond with the temperature data used in the analysis)
- species for which records indicated a flowering season longer than three months
- species represented by ten or less independent specimen records.

This process of elimination reduced the original 171 to a working list of 20 species representing 7 families. The metric chosen for analysis was the onset of flowering and referred to as ‘flowering observation’. This approximation was extracted from the data by discarding all but the earliest collection date for any one species in any one flowering season. For analysis, flowering observation was expressed as a Julian date (January 1st = day #1), adjusted to overcome the difficulty of dealing with plants whose flowering season spanned the transition from one year to the next.

Data obtained from the Australian Bureau of Meteorology provided evidence of an increase in the mean annual temperature of 0.74°C over the 1950-2007 period, and by matching spatial locations of herbarium specimens to the temperature data and using ordinary least-squares linear regression models, eight of the 20 species were identified for which flowering response showed a significant negative relationship with mean annual temperature (i.e. a temperature increase at the point of collection corresponded with earlier flowering observations for these species). One of these species was *Prasophyllum tadgellianum* ( $R^2 = 0.63$ ,  $P < 0.0001$ ;  $y = -8.99x + 447.62$ ), one of two orchids whose sister species *P. suttonii*, was the only species to show a positive correlation ( $R^2 = 0.17$ ;  $P = 0.06$ ;  $y = 3.73x + 375.56$ ). Simultaneous convergence/divergence in flowering times between these two closely related species in response to the same changing conditions may have long-term implications for hybridization (see also case study 2 “Tracking phenological shifts and evolutionary impacts relating to climate change” below). Of the eight species, only one, *Senecio pectinatus* var. *major*, was deemed suitable by the investigators for use as an indicator species for environmental monitoring.

With only one potentially suitable species selected from an original 171 candidates, the attrition rate was high, and it becomes clear that the search for a range of site-specific indicator species requires interrogation of large data bases. This would hardly be possible without the availability and convenience of the rich resources provided by herbaria. With a realization of the emerging importance of their role in climate change research, it is to be hoped that increased funding is forthcoming for the continued collection and curation of the voucher specimens upon which these data sets are based. Gallagher, Hughes et al. (2009) concluded that “*the real utility of herbarium specimens lies in providing a complementary historical baseline of data to which new field-based observational records can be compared*” p 7.

### ***Case Study 2: Tracking phenological shifts and evolutionary impacts relating to climate change***

Shifts in phenology impact on community-level interactions and other evolutionary processes, and have the potential to induce ecosystem compositional turnover as the synchrony between developmental times of co-evolved and dependent species is disrupted (Beaubien and Freeland 2000). For plants such as orchids these shifts may impinge on the finely tuned relationships with their pollinators providing opportunities for interspecific hybridization.

The present case study investigation has two aspects. As for the previous study, the first component incorporates an examination of herbarium collections in a search for evidence of recent shifts in flowering phenology in line with recent climate change. The species chosen for the study belong to the genus *Diuris* Sm. (donkey orchids), a genus of 64 Australian species and four named hybrids, as listed by Jones (2006). The flowers have a structural resemblance to those of the pea family (Fabaceae), considered by many to be a mimicry associated with the deceptive attraction of native bee pollinators (Indsto, Weston et al. 2007). Natural hybridization is common within this genus. In South Australia, co-occurring local populations of *D. orientis* (Fig. 3), *D. behrii* and *D. pardina* readily form hybrids exhibiting considerable variation in floral colour patterns. *Diuris* X *palachila*, a fertile, natural hybrid between *D. pardina* and *D. behrii* is capable of producing further

crosses between either parent and the hybrid species itself, resulting in a confusing array of morphologically different forms (Jones 2006). Orchid plants arise from underground tubers towards the end of winter, and flowering finishes before hot summer temperatures become established. The flowering period is relatively short and compact thus making it easier to detect shifts over time (Rumpff, Coates et al. 2008). Spotting and photographing orchid flowers is a very popular pastime, and many dedicated and knowledgeable observers have amassed valuable photographic collections to supplement the considerable collection held in the state herbarium.

Presented in this section is an initial exploratory analysis of the data extracted from the AVH electronic database, using simple linear regression (SLR). This amounted to 388 individual South Australia records of the study species, *D. orientis*, *D. behrii* and *D. pardina*, from a total of 6395 Australian *Diuris* records. All specimens are represented by one and only one record eliminating the need to check for, and remove, duplicates. Converting the collection date format to yyyy/mm/dd makes for easier sorting and eliminates the problematic issue of mistaken century of collection. Of the 388 records, 72 were discarded because collection date was not provided, leaving 316 usable records available for analysis. Two hundred and nine of the total *Diuris* data set had no information on their spatial coordinates, but as many of these were also undated this omission did not necessitate further reduction. The following analysis discussed in this chapter has been restricted to the two species, *D. orientis* and *D. behrii*<sup>2</sup>.

An assumption was made that collection date was a reliable surrogate for peak flowering, and where multiple records existed for any one flowering season, the median value was chosen as the most appropriate measure for peak flowering. For analysis, date of peak flowering was converted to the number of days following the winter solstice, and calculated from June 22 (day #1). This not only keeps the metric within a reasonable numerical range, but also conveniently differentiates lengthening day from shortening day (with negative values) flowering responses. For these species flowering occurs during the noticeably lengthening days from late winter to late spring.

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<sup>2</sup> Analysis of *D. pardina* proved inconclusive

## Initial exploratory data analysis

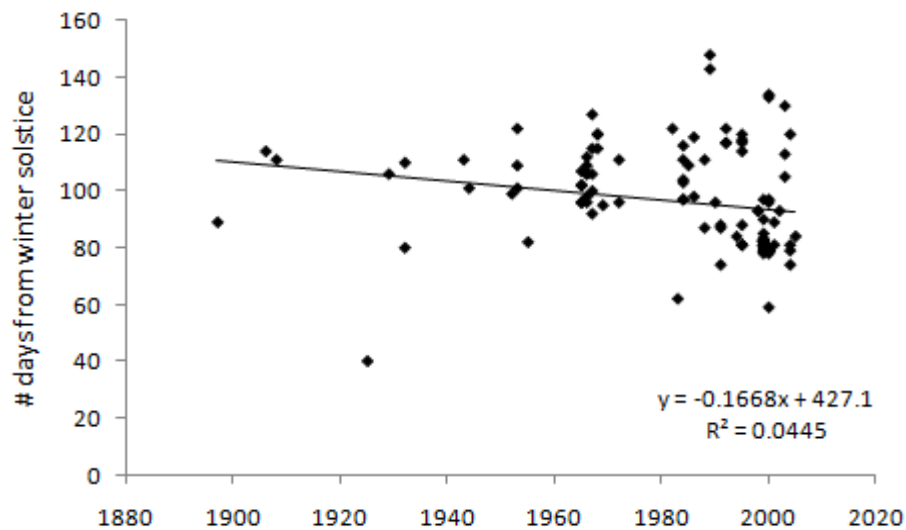
### *Simple linear regression*

Methodologically, the detection of trends in time series is frequently performed via the classical statistical methods using slopes of linear regression models where phenological dates are plotted against time (Bradley, Leopold et al. 1999; Menzel and Fabian 1999; Schwartz and Reiter 2000; Defila and Clot 2001; Menzel, Estrella et al. 2001; Ahas, Aasa et al. 2002; Peñuelas, Filella et al. 2002b; Menzel 2003) and, more rarely, by other curve fitting methods (Ahas 1999; Sagarin and Micheli 2001). For herbarium applications see Table 1 and for general phenology applications see Menzel and Fabian (1999), Walther, Post et al. (2002), Parmesan and Yohe (2003), Root, Price et al. (2003), and Menzel, Sparks et al. (2006).

The slope of the linear regression equation then indicates the average rate of change in phenology given as days per annum (days/a), or temperature expressed as degrees per annum ( $^{\circ}\text{C/a}$ ). The major disadvantages of this least squares approach (SLR) is its limitation to time series with a comparatively linear trend, possible poor extrapolation properties, and sensitivity to outliers (or so-called extremes (Schlittgen and Streitberg 1999)) and to boundary values. As noted by von Storch and Zwiers (2001), one or two outliers can seriously distort the results of a least squares analysis (NIST/SEMATECH, e-Handbook of Statistical Methods, 2006, available at <http://www.itl.nist.gov/div898/handbook/index2.htm>).

A plot of the entire data set for *D. orientis* spanning a 98 year period from 1897-2005 (Fig. 4) supplies provisional evidence of a shift to earlier flowering of between one to two days per decade, or approximately 16 days over the entire period. This figure also clearly illustrates the non-uniform nature of the data series and identifies possible collection bias with a concentration of points for the period 1965-1969 and again around 2000. This recent increase in collection effort does not appear to substantiate the claims made by (Prather, Alvarez-Fuentes et al. 2004; Lavoie and Lachance 2006) of a growing decline in collection activity. However, orchids species have popular appeal and have been the focus

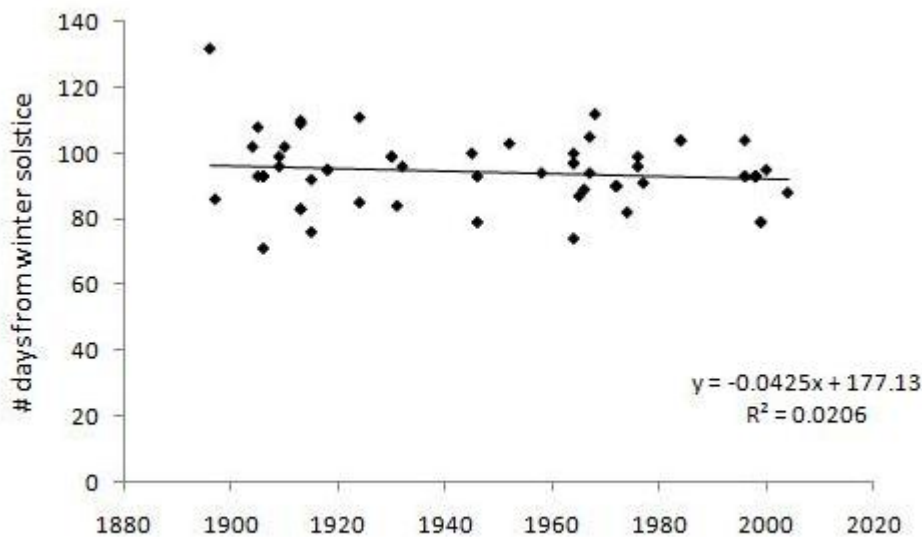
of many recent surveys. A closer investigation of the data reveals that the orchid specialist and author, D.L. Jones, collected all 24 specimens recorded for the period between 7th and 14th September 1999, confirming dependence and necessitating their exclusion from the final analysis. Following substitution of all multiple data points for any one season <sup>3</sup> by their median value, and discarding the outlier in 1925, former evidence of a negative trend (i.e. towards earlier flowering) diminished ( $y = -0.1058x + 308.76$ ,  $R^2 = 0.0397$ ), and the mean number of days to peak flowering across all latitudes increased from 96 to 100 days.



**Fig. 4** Simple linear regression plot of all data points for *D. orientis* spanning the period 1897-2005. The concentration of collection points around 1965-1969 and around 2000 may introduce collection bias.

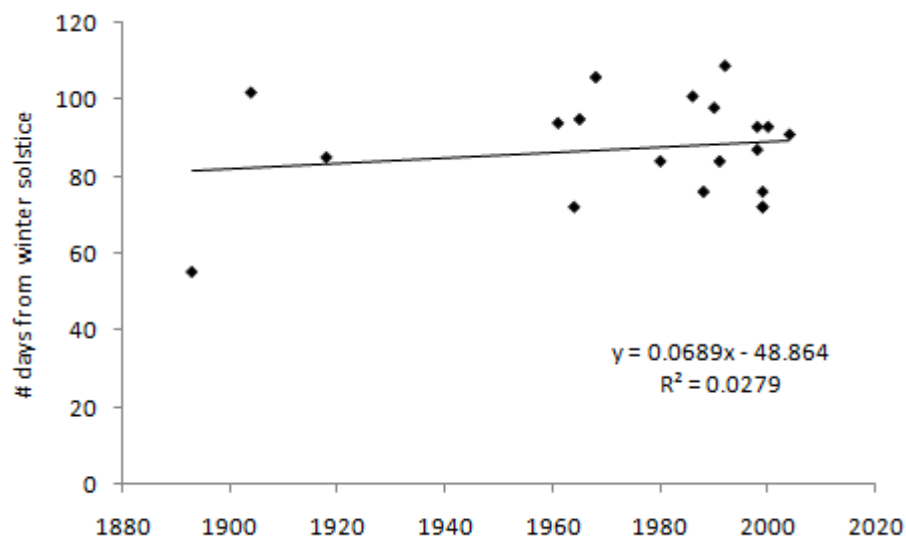
<sup>3</sup> Including those of D.L. Jones

Because temporal changes are not always consistent across the entire range of a species (Lavoie and Lachance 2006) the possible effect of latitude was investigated. When regions are considered separately as central (Fig. 5a) and northern (Fig. 5b), there emerges slight evidence of a difference in mean peak flowering date of *D. behrii* populations, and tendency for an opposite trend in flowering time shifts over this period. Southerly populations have flowered on average seven days later than more northerly populations, where hotter and drier conditions are more likely to induce earlier flowering. However, these regional differences appear to be diminishing as a result of the contrary shifts. See Gallagher, Hughes et al. (2009) above for further evidence of opposite shifts in flowering time for *Prasophyllum* species.



**Fig. 5a** Number of days to peak flowering for *D. behrii* for  $-34.5^{\circ} < \text{latitudes} < -35.5^{\circ}$ ; average of 94 days to flowering over the period. The negative slope indicates slight evidence of a trend to earlier flowering.





**Fig. 5b** Number of days to peak flowering for *D. behrii* north of latitude  $-34.5^{\circ}$ ; average of 87 days to flowering over the period. The positive slope provides some indication of a trend to later flowering.

## Limitations of standard methods to detect trend

### *Non-linearity of trends*

As noted by Hudson, Keatley et al. (2005), Sparks, Jeffree et al.(2000), Dose and Menzel (2004) and Parmesan (2007), this commonly used method of searching for signals in phenological time series presents problems. Indeed it will be difficult to find a linear model that fits the data well for essentially non-linear processes. This is true particularly as the range of the data increases (Schleip, Menzel et al. 2008). Importantly, Menzel, Estrella et al.(2008) noted that when utilising SLR, the length of a time series and its start and end dates are crucial in correct detection of changes, and in estimating their magnitude. This is particularly so when highly variable, multi-decadal, phenological time series are analysed (Dose and Menzel 2004). We show this also for the three herbarium data sets analysed here.

How can we accommodate for non-linear responses of phenology to time (year) and/or to climatic factors? In Chapter 20 (Hudson 2010b) on meta analysis this question is

addressed by the illustration of three approaches to modelling phenological time series assuming possible non-linear trends, namely: The Generalized Additive Model for Location, Scale and Shape (GAMLSS) approach (Rigby and Stasinopoulos 2005; Stasinopoulos and Rigby 2007) applied recently to flowering records of four Eucalypts (*Eucalyptus leucoxylon*, *E. microcarpa*, *E. polyanthemos* and *E. tricarpa*), from Maryborough, Victoria, Australia, for the period 1940 – 1971 (Hudson, Rea et al. 2008; Hudson, Kim et al. 2009); see also Chapter 10 (Hudson, Kim et al. 2010).

1. Penalized spline (*P*-spline) signal regression (PSR) (Marx and Eilers 1999; 2005) applied recently to a dataset of 208 species compiled from the 1978 to 2001 flowering records of the Last family (Last, Roberts et al. 2003; Roberts 2008), and
2. The Bayesian nonparametric function estimation approach of Dose and Menzel (2004) applied to blossom (onset) time series of *Prunus avium* L., *Galanthus nivalis* L. and *Tilia platyphyllos* SCOP (1896–2002) in Germany, in an analysis of the variations of the onset of phenological phases in the 20th century.

In this chapter we present the GAMLSS approach to show its greater accuracy and relevance to the assessment of non-linear trends over time (year) for the herbarium records. We clearly establish the existence of non-linear trends in days to peak flowering of herbarium records. A short introduction to GAMLSS follows, as does a brief cautionary point on change points in time series analysis and detection.

It is noteworthy that GAMLSS models often provide the researcher with a visual indication of points of change in a given time series. However, formal tests of significance for change points should be carried out in addition to fitting GAMLSS models to phenological time series. We explore the use of both non-linear methods (GAMLSS) and change points methods in combination in this analysis of herbarium records. It is important to note (as evidenced in the *D. behrii* for  $-34.5^{\circ} < \text{latitudes} < -35.5^{\circ}$  data) that whereas GAMLSS modelling of a cubic spline or non-linear effect of year (time) may show statistically non-significant effects of year; formal change point detection tools can point to significant and abrupt change points.

*Need for formal change point analyses*

Rapid shifts in climate can lead to, or be contemporaneous with, abrupt phenological changes. Although it has been appreciated for some time that these change points cannot be detected satisfactorily (if at all) by either regression or correlation methods, they continue to be used for the detection of temporal changes in phenology (Keatley, Fletcher et al. 2002; Hudson, Keatley et al. 2005; Cleland, Chiariello et al. 2006; Cleland, Chuine et al. 2007). However, new techniques for change point analysis which enable a quantitative representation of non-linear phenological responses and associated (abrupt and non-abrupt) rates of change have been reported (Hudson, Barnett et al. 2003; Sparks and Tryjanowski 2005; Keatley, Chambers et al. 2008; Keatley and Hudson 2008). See also Bayesian change point techniques (Hasselmann 1998; Dose and Menzel 2004; Menzel and Dose 2005; Menzel, Estrella et al. 2008; Schleip, Rutishauser et al. 2008). These allow for the analysis of so-called change-point probabilities which provide the researcher with the ability to both visualize and quantify major changes (shifts) in long-term time series. It is anticipated that future developments in change point analysis will address the assessment of increasingly complex time series models such as multiple change-point models.

In this chapter a model-free method of change-point detection has been used to establish the existence, or otherwise, of significant change points in time series of days to peak flowering of herbarium records (Moskvina and Zhigljavsky 2003). This method is based on the sequential application of singular-spectrum analysis (SSA) (Chapter 18) (Hudson and Keatley 2010b) to subseries of the original series, and the monitoring of quality of approximation of the other parts of the series by suitable approximates (Moskvina and Schmidt 2003). The three test data sets obtained from AVH, namely flowering dates of South Australian orchid species *Diuris behrii* ( $-34.5^{\circ} < \text{latitude} < -35.5^{\circ}$ ), *D. behrii* (latitudes  $< -34.5^{\circ}$ ) and *D. orientis* (all latitudes) are used as exemplars. We show that significant change points exist for two of the three herbarium series; change points which could not be detected using traditional linear regression analysis.

## Introduction on GAMLSS

The Generalized additive model for location, scale and shape (GAMLSS) is part of the Generalized Linear Model (GLM) (Nelder and Wedderburn 1972) and the Generalized Additive Model (GAM) ‘family’ (Hastie and Tibshirani 1999; Hastie 2008). GAMLSS was introduced by Rigby and Stasinopoulos (2001) and Akantziliotou, Rigby et al. (2002), and further developed by Rigby and Stasinopoulos (2005) to overcome various limitations of the popular GLM and GAMs. For example, GAMLSS can deal with non-normally distributed data (e.g. highly skewed, or kurtotic continuous and discrete distributions). Current updates of GAMLSS can deal with up to 50 different types of distributions (Stasinopoulos and Rigby 2007). See Chapter 10 (Hudson, Kim et al. 2010) for mathematical theory and details on the implementation of GAMLSS.

Importantly, the GAMLSS family extends linear regression to a non-linear form by allowing each regression variable to have a non-linear relationship with the dependent variable. The type of non-linearity is not pre-specified *a priori*, but is calculated and tested for in the modelling process. GAMLSS models are thereby a general framework for univariate regression analysis which allow for testing of semi-parametric models. By semi-parametric, we mean they need a parametric distribution for the response variable, although they can cope with a wide range of distributions such as the Poisson, negative binomial, log normal, Weibull etc. These GAMLSS models are thus ‘semi’ in the sense that the modelling of the actual parameters, such as the mean or location (as functions of the explanatory variables), may involve using non-parametric smoothing functions, such as for example cubic smoothing splines [cs()]. For a discussion on cubic splines see Chapter 12 (Roberts 2010).

The benefits of GAMLSS for phenological and herbarium time series data are that they:

1. can identify the potential drivers <sup>4</sup> of the event of interest from a multiplicity of predictors such as climate and food sources etc.,
2. allow for non-linear impacts of time and/or the explanatory variables or predictors,

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<sup>4</sup> They do not, however, distinguish cause and effect

3. can statistically detect thresholds; for example, the lowest temperature for the commencement of flowering and
4. can model and/or account for the auto-correlated nature of the phenological series; for example by incorporating lag effects, such as autoregressive AR(k) lags ( $k=1,2,\dots$ ).

Recent applications of GAMLSS have involved modelling climate with Sudden Infant Death Syndrome counts (Hudson, Rea et al. 2008) and in the establishment of the world standard child growth curves by the World Health Organisation (Borghi, de Onis et al. 2006). Further examples are given in Rigby and Stasinopoulos (2005) and Stasinopoulos and Rigby(2007). As far as the authors are aware, GAMLSS have not been applied to phenological research to date, apart from a forthcoming paper by Hudson, Kim et al. (2009). It should also be noted that Hudson, Barnett et al. (2003) used GAMs (the precursor to GAMLSS) and Bayesian methods to model *E. leucoxydon* flowering, and found that the estimated effects of mean temperature were smoothly non-linear on flowering intensity. The work of Roberts (2008) and Chapter 12 (Roberts 2010) on penalized splines (*P*-splines) (Eilers and Marx 1996) illustrates a method that also has some inter-relatedness with GAMLSS in that spline functionals are used in the modelling (the term "spline" refers to a wide class of functions that are used in applications requiring both data interpolation and/or smoothing).

### ***GAMLSS Methods***

The GAMLSS framework of statistical modelling is implemented in a series of packages in R (R Development Core Team 2007), a free software (see URL <http://www.R-project.org>). The packages can be downloaded from the R library, [CRAN](http://cran.r-project.org/web/packages/) ([cran.r-project.org/web/packages/](http://cran.r-project.org/web/packages/)), or from <http://www.gamlss.com>. For this study the GAMLSS procedure was used with a cubic spline smoothing function (Stasinopoulos and Rigby 2007). Each model assumes that the flowering series represents normally distributed data and the RS algorithm, a generalization of the algorithm of Rigby and Stasinopoulos (1996), was used to obtain the estimates of the year (time) effect.

### ***GAMLSS and Change point results***

Table 5 shows GAMLSS modelling of the effect of year (time) on the number of days to peak flowering of *D. orientis* spanning the period 1897-2005. The significant and negative cubic spline effect of year (cs(year)) of -0.1668, with an associated non-linear P value of 0.0009, indicates a highly significant non-linear and overall negative trend with year for *D. orientis*. Figure 6 indicates the curvilinear nature of change in days to peak flowering for *D. orientis* (1897-2005). A significant change point in the time series in 1925, marks the end of a period of decreasing trend (to earlier flowering). This is followed by a period of increasing trend to 1972, after which a slow negative trend till 1984 becomes increasingly negative (significant change point or deviation starting about 1985) and most negative, indicating significantly earlier flowering, after the change point in 1995.

Note that the precursor to GAMLSS, namely GAMs, gave a  $R^2$  for the model of 17.63% (compared to the  $R^2$  of 4.45% obtained by the earlier simple linear regression (SLR) analysis Fig. 4), indicating that the non-linear cubic spline model fits the data substantially better than that achieved by SLR. The scaled deviance statistics (Stasinopoulos and Rigby 2007), the Akaike information criterion (AIC) (Akaike 1983) and the Schwarz Bayesian Criterion (SBC) (Stasinopoulos and Rigby 2007) goodness of fit statistics are also shown for the resultant GAMLSS model in Table 6. The sparsity of data prior to 1925 may, however, be biasing the GAMLSS analysis prior to 1925.

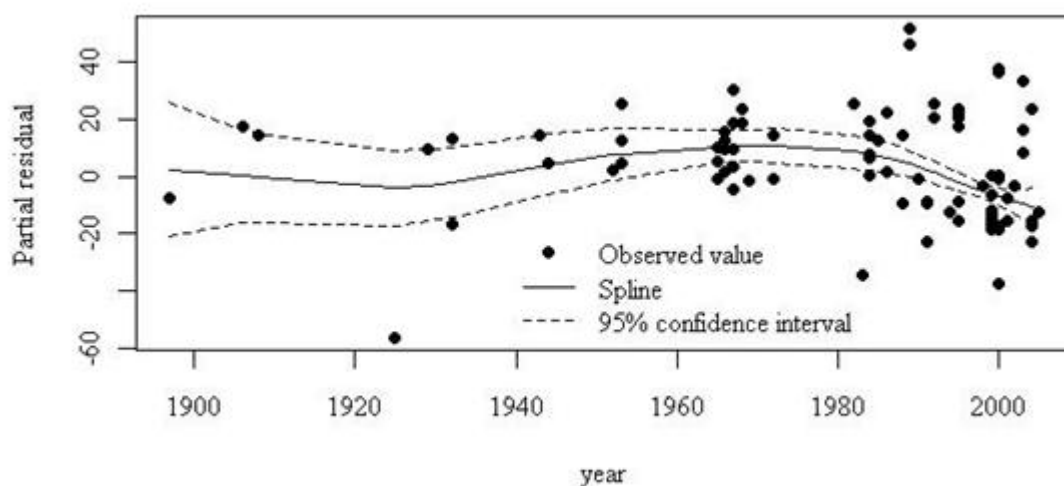
**Table 5.** Intercept and year effects via GAMLSS modelling of the number of days to peak flowering of *D. orientis* spanning the period 1897-2005.

	GAMLSS	S.E.	t value	P value	GAMLSS	S.E.	t value	Non linear	GAMs
	intercept				cs(year)			P value	100(R <sup>2</sup> )%
<i>D. orientis</i>	427.10	134.520	3.175	0.001	-0.167	0.068	-2.459	0.0009	17.63

**Table 6.** Significant change points in the time series of the number of days to peak flowering of *D. orientis* spanning the period 1897-2005; with GAMLSS goodness of fit statistics (GD, AIC and SBC) shown. GD denotes the scaled deviance statistic (Stasinopoulos and Rigby 2007); AIC denotes the Akaike information criterion (Akaike 1983) and SBC denotes the Schwarz Bayesian Criterion (Stasinopoulos and Rigby 2007) goodness of fit statistics.

	Change point years: Moskvina & Zhigljavsky (2003)	Global Deviance(GD) <sup>ϕ</sup>	AIC <sup>ϕ</sup>	SBC <sup>ϕ</sup>
<i>D. orientis</i>	1925, 1972, 1984, 1995	967.2	979.2	995.6

□

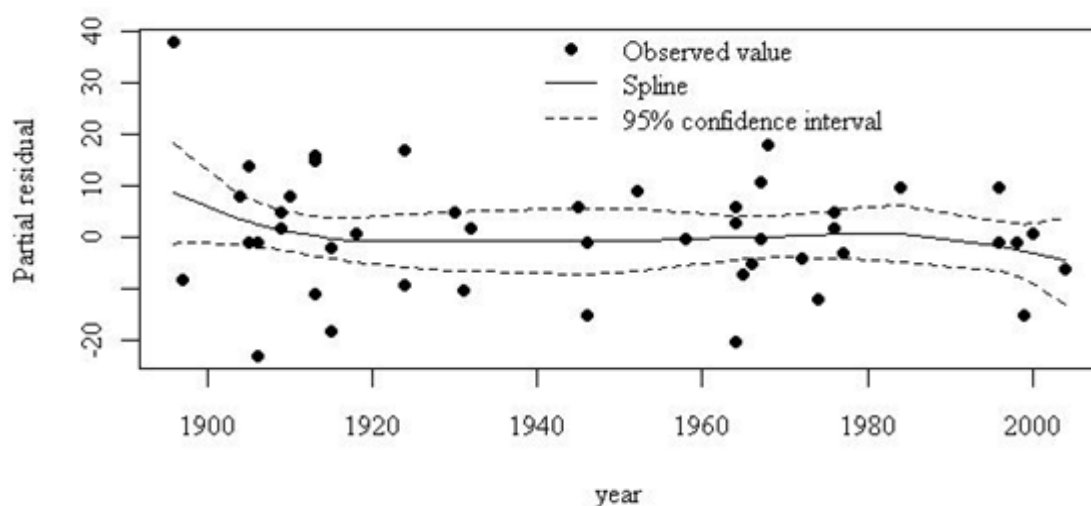


**Fig. 6** Variant of Figure 4: GAMLSS term plot of all data points for *D. orientis* spanning the period 1897-2005; showing the cubic spline effect of year (cs(year)) and its associated 95% confidence interval. [Spline line and 95% confidence band for effect of year flowering day number for *D. orientis*]

Table 7 shows the GAMLSS model for the effect of year (cs(year)) on the number of days to peak flowering of *D. behrii* between the latitudes  $-35.5^0$  and  $-34.5^0$  for the period 1896-2004. The cubic spline effect of year (cs(year)) of -0.0425 is, however, not statistically significant. Figure 7 indicates the relatively flat profile of the days to peak flowering for *D. behrii* after 1924; with a slight increase to 1964 and then a negative trend post 1984 (as for *D. orientis*).

It is noteworthy that whilst the GAMLSS cubic spline effect for year (cs(year)) is not statistically significant, a formal change point detection analysis gives three years of significant change (Table 8). In summary, from Table 8 and following the change point detection methods of Moskvina and Zhigljavsky (2003), significant change points in the *D. behrii* for  $-34.5^0 < \text{latitudes} < -35.5^0$  time series were found at 1924 (a decrease), 1964 (increase) and at 1999 (decreased trend). Recall that there was a change point (decrease) in 1925 for the days to peak flowering of *D. orientis* (1897-2005), similar to the *D. behrii* for  $-34.5^0 < \text{latitudes} < -35.5^0$  time series. The years 1999 and 1995 mark the beginning of an increased negative trend for the *D. behrii* (for  $-34.5^0 < \text{latitudes} < -35.5^0$ ) and the *D. orientis* time series, respectively (an earlier point of decrease could not be detected for the *D. behrii* data, due to lack of records after 1984 and 1998). GAMs gave a  $R^2$  for the model of 7.11% (in contrast to the  $R^2$  of 2.06% obtained by the earlier SLR (see Fig. 5a). GD, AIC and SBC goodness of fit statistics are shown for the resultant GAMLSS model in Table 8.





**Fig. 7** Variant of Figure 5a: GAMLSS term plot of data points for *D. behrii* number of days to peak flowering for *D. behrii* for  $-34.5^{\circ} < \text{latitudes} < -35.5^{\circ}$ ; mean over the period of 94 days; showing the cubic spline effect of year (cs(year)) and its associated 95% confidence interval.

**Table 7.** Intercept and year effects via GAMLSS modelling of number of days to peak flowering for *D. behrii* for  $-34.5^{\circ} < \text{latitudes} < -35.5^{\circ}$ ; mean over the period of 94 days spanning the period 1896-2004.

	GAMLSS intercept	S.E.	t value	P value	GAMLSS cs(year)	S.E.	t value	Non linear P value	GAMs 100(R <sup>2</sup> )%
<i>D. behrii</i> (all)	177.131	71.485	2.478	0.0163	-0.0425	0.037	-1.149	NS*	7.11

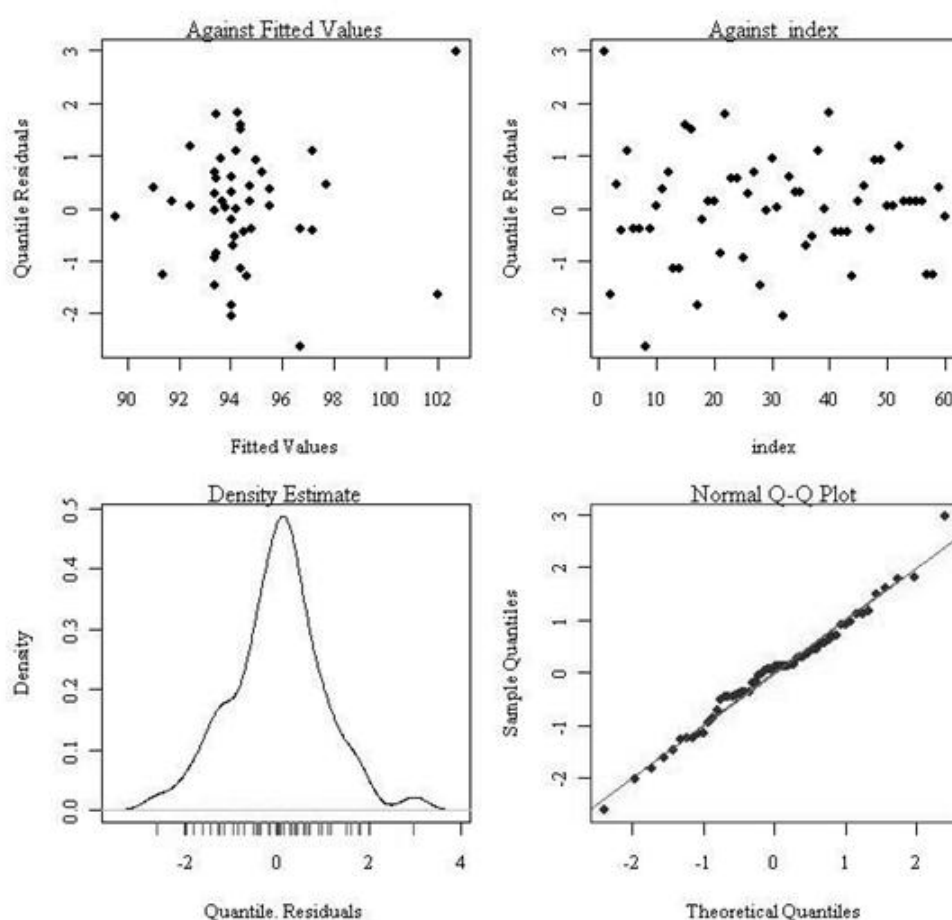
\*NS= not significant

**Table 8.** Significant change points in the time series of number of days to peak flowering of *D. behrii* for  $-34.5^{\circ} < \text{latitudes} < -35.5^{\circ}$ ; mean over the period of 94 days spanning the period 1896-2004; with GAMLSS goodness of fit statistics (GD, AIC and SBC) shown.

	Change point years: Moskvina & Zhigljavsky (2003)	Global Deviance(GD) <sup>φ</sup>	AIC <sup>φ</sup>	SBC <sup>φ</sup>
<i>D. behrii</i> (all)	1924, 1964, 1999	444.5	456.5	469.1

<sup>φ</sup> GD denotes the scaled deviance statistic (Stasinopoulos and Rigby 2007); AIC denotes the Akaike information criterion (Akaike 1983) and SBC denotes the Schwarz Bayesian Criterion (Stasinopoulos and Rigby 2007) goodness of fit statistics.

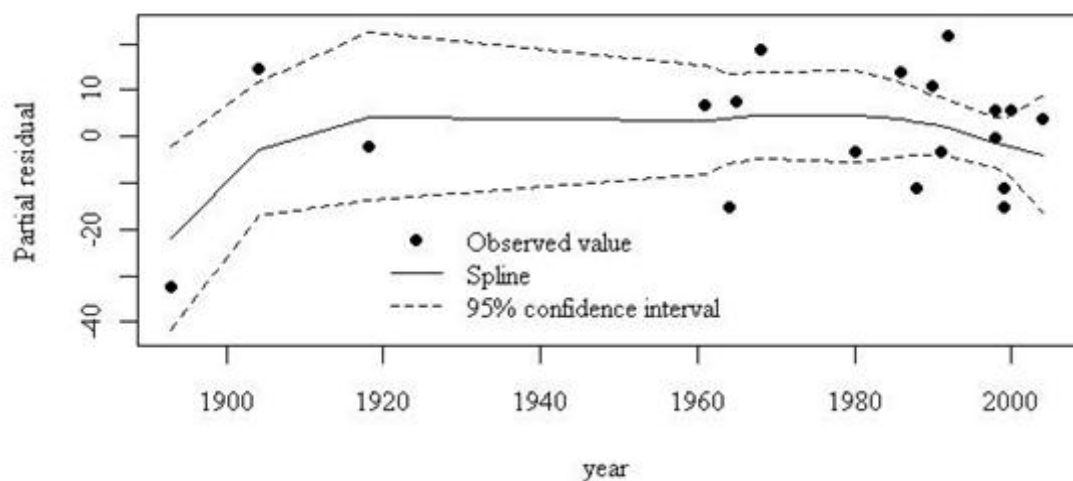
Diagnostic plots (Fig. 8) of quantile residual diagnostics and a normal Q-Q plot of the theoretical quantiles show that the GAMLSS intercept and cubic spline year (time) effect model fits the data well. This is indicated by the straight 1:1 line for the sample versus theoretical quantile plot (RHS bottom). For brevity we show only this model's diagnostic plots.



**Fig. 8** Diagnostic plots of GAMLSS analysis of the number of days to peak flowering for *D. behrii* for  $-34.5^{\circ} < \text{latitudes} < -35.5^{\circ}$ ; mean over the period of 94 days; showing quantile residual diagnostics, and normal Q-Q plot of the theoretical quantiles. A straight 1:1 line (plot on RHS bottom) indicates a good fit of the GAMLSS model.

Table 9 shows GAMLSS modelling of the number of days to peak flowering of *D. behrii* north of latitude  $-34.5^0$  for the period 1893-2004. Interestingly the cubic spline effect of year (cs(year)) is positive with a value of 0.0689; but this is not a statistically significant positive trend. Figure 9 indicates a relatively flat profile for the days to peak flowering for *D. behrii* after 1918; with slightly later peak flowering day after 1964 and earlier time to peak flowering post 1999 (although this trend is non-significant). Here the GAMLSS cubic spline effect for year (cs(year)) is not statistically significant and likewise a formal change point detection found no significant points of change (Table 10).

GAMs gave a high  $R^2$  for the model of 25.71% (in contrast to the  $R^2$  of 2.79% obtained the earlier SLR analysis (see Fig. 5b).



**Fig. 9** Variant of Figure 5b: GAMLSS term plot of number of days to peak flowering for *D. behrii* north of latitude  $-34.5^0$ ; mean 87 days; showing the cubic spline effect of year (cs(year)) and its associated 95% confidence interval. [Spline line and 95% confidence band for effect of year flowering day number for *D. behrii*.]

**Table 9.** Intercept and year effects via GAMLSS modelling of number of days to peak flowering for *D. behrii* north of latitude  $-34.5^{\circ}$ ; mean 87 days (1893-2004). \*NS= not significant

	GAMLSS intercept	S.E.	t value	P value	GAMLSS cs(year)	S.E.	t value	Non linear P value	GAMs 100(R <sup>2</sup> )%
<i>D. behrii</i>	-48.864	154.050	-0.317	NS*	0.069	0.078	0.884	NS*	25.71

**Table 10.** Significant change points in the time series of number of days to peak flowering of *D. behrii* north of latitude  $-34.50$ ; mean 87 days; with GAMLSS goodness of fit statistics (GD, AIC and SBC) shown. GD denotes the scaled deviance statistic (Stasinopoulos and Rigby 2007); AIC denotes the Akaike information criterion (Akaike 1983) and SBC denotes the Schwarz Bayesian Criterion (Stasinopoulos and Rigby 2007) goodness of fit statistics.

	Change point years: Moskvina & Zhigljavsky (2003)	Global Deviance(GD) <sup>ϕ</sup>	AIC <sup>ϕ</sup>	SBC <sup>ϕ</sup>
<i>D. behrii</i>	No significant change points	153.6	156.5	171.6

## Discussion

GAMLSS modelling has shown a significant curvilinear advance in days to peak flowering for the *D. orientis* records covering the period 1897-2005. The change point year of 1972 coincides with an abrupt change in the El Niño Southern Oscillation (ENSO), from an extended cold La Niña phase to one dominated by El Niño (Wolter and Timlin 1993; 1998) (Fig. 1). The negative trend beginning around 1972 (Fig. 6) coincides with the warm El Niño phase and the period of significant warming in Australia from the mid 1980s when some of the warmest years on record were experienced <http://www.bom.gov.au/climate/change/amtemp.shtml>. The change point years of 1984 and 1995 come at the end of the most extreme (1982-83), and the most protracted (1990-1995), El Niño events on record (Fig. 1).

It is noteworthy that a change point or deviation starting about 1985 was also shown in the study of Dose and Menzel (2004). These authors developed nonparametric regression

from a Bayesian viewpoint to explore the possible non-linear functional behaviour of blossom onset time series of *Prunus avium* L., *Galanthus nivalis* L. and *Tilia platyphyllos* SCOP (1896–2002) in Germany, and thereby analysed the variations of the onset of phenological phases in the 20th century. The period covered in their study is similar in to that represented by the herbarium records for *Diuris* (1896-2005) analysed here.

Dose and Menzel (2004) trialled three different models: a constant model, a linear model (though more complex than SLR) and a one change point model, to represent the functional behaviour of their blossom onset time series. See Dose and Menzel (2004; 2006) for details on the relevant computational and mathematical formulae. Further discussion appears in Chapter 11 (Schleip, Menzel et al. 2010). However, the change point methods used here, following the development of Moskvina and Zhigljavsky (2003), allow for multiple change points in a given time series, not just one change point as in Dose and Menzel (2004).

Dose and Menzel (2004) calculated the rates of change (days per year) as well as the so-called average functional behaviour with its associated uncertainty range. This model average for the rate of change was calculated from the mean rates of change of the three models, weighted by their respective probabilities. The rate of change was shown to be essentially zero over most of the century, with a significant and abrupt change point or deviation starting around 1985 (2004), and a current rate of change of -0.6 days per year (Dose and Menzel 2004). This negative curvilinear slope and associated rate of change was associated with an uncertainty of -0.5 days per year. Likewise, GAMLSS modelling and change point analysis of the days to peak flowering for *D. behrii* indicate a relatively flat profile from 1924 to 1984, and an estimated rate of change of -0.7 days per year for *D. orientis* and -0.8 days per year for *D. behrii*, obtained from SLR slopes for the same current period, thus comparing favourably with the Dose and Menzel (2004) estimates.

The non-uniform periods of change that typify the climate of the 20th century (Dose and Menzel 2004; Rutishauser, Luterbacher et al. 2007; CSIRO 2008) pose a particular challenge when linear regression analysis is used for the reconstruction of trends. To

properly address the question of change, periods of no change must be considered as an important part of the overall picture. While it is obviously necessary to determine changes in phenology with respect to corresponding changes in climate, it is likewise, but maybe less obviously, important to determine events throughout periods of reasonably stable conditions.

Schleip, Menzel et al. (2008) also applied linear trend analysis and Bayesian model comparison to an investigation of three unique, 250-year phenological time series from Switzerland and France, spanning the years from 1753 to the present (Chuine, Yiou et al. 2004; Schleip, Menzel et al. 2006; Meier, Rutishauser et al. 2007). Schleip, Menzel et al. (2008) detected major changes in both long-term phenological and temperature time series at the end of the 20th century, as we do here for the herbarium flowering records. It is noteworthy that Schleip, Menzel et al. (2008) showed that the functional description of the change-point model exhibited a sharp decline at the end of the 20th century for all the phenological time series studied. In this chapter we also report change points of increased decline near the end of the 20th century, namely near 1995 and 1999/2000 for *D. orientis* and *D. behrii* respectively. Analysis of change incorporating temperature is a topic of further investigation.

Whilst GAMLSS splines often give the researcher a visual indication of points of change in a time series, formal tests of significance of change points should be carried out in addition to fitting GAMLSS (splines or polynomials approaches) to phenological time series. We advocate the combined use of both non-linear methods (GAMLSS) and change points methods in the analysis of herbarium records. It is important to note that whereas GAMLSS modelling of a cubic spline or non-linear effect of year (time) may show statistically non-significant effects of year, formal change point detection tools can point to significant abrupt change points, as evidenced in the *D. behrii* for  $-34.5^{\circ} < \text{latitudes} < -35.5^{\circ}$  data (Table 8). Linear regression methods cannot accommodate significant and abrupt change points; although piecewise linear regression methods may. Such an approach (but via Bayesian methods) was used in the seminal paper of Dose and Menzel (2004) in their linear model approach.

While climate change forecasts provided by the Bureau of Meteorology <http://www.bom.gov.au/climate/change/amtemp.shtml>, indicate that the current trend of persistent and substantial warming which began in 1950 will continue into the future, orchid species emerge at the end of a generally mild, and hopefully wet, winter and finish flowering before summer heat sets in. Future refinements to the GAMLSS modelling of these herbarium records will incorporate both temperature and rainfall as predictors, despite the inherent complexities of irregularly spaced time points, the inclusion of lagged dependencies of current with past climate and/or flowering days, for up to 12 months prior to the event; and the development of specific functionals for the herbarium flowering records. Sparsity of data and irregularity of records over time, as well as the need for more complex underlying distributions, remains an issue in the analysis of trends in herbarium records.

### *Potential for change in hybridization dynamics*

Understanding the impact of climate on the phenology of these orchids, in combination with knowledge of their genetic heritage, will help clarify their future prospects for survival, both as populations and species. Opportunities for pollination will be diminished if the synchrony between plants and their pollinators is disrupted, especially when the pollinators themselves are seasonal and impacted by climate change (Fitter and Fitter 2002). On the other hand, seed predation may be disrupted if the phenology of seed predators shift independently of the timing of seed production (Tarayre, Bowman et al. 2007). Thus when sympatric species display divergent responses to change, the resulting mistiming of seasonal activities has the potential to disrupt existing biotic interactions (Walther, Post et al. 2002; Visser and Both 2005).

The historical baseline data forthcoming from this investigation should be well suited to place the complementary phase of this study in perspective. This will entail field and molecular investigations to determine the genetic variation present within sympatric populations of these orchid species and the extent of natural hybridization occurring among them. Flowering time has a genetic basis incorporating levels of individual

variation within populations as a form of bet-hedging against normal patterns of year to year variability. Shifts in phenology as a consequence of climate change are expected to have a number of major impacts on biological systems, and successful evolutionary adaptation is more likely when high levels of genetic variance (Fox 1990; Franks, Sim et al. 2007) provide the resources for a species to cope with change in the short term, outcompete less resilient species and have better prospects for long-term survival (Cavers, Navarro et al. 2003; Burke 2004). Species which do not have sufficient capacity to respond to the conditions imposed by enhanced and rapid change will be prone to local extinction.



### **Chapter 3 Seasonal temperature and rainfall as drivers of change in flowering phenology of the Australian terrestrial orchid, *Diuris* (Orchidaceae): analysis of herbarium records 1893 to 2005**

This paper has been prepared as a submission for publication in *Climatic Change*

Phyllis F. MacGillivray

*School of Earth and Environmental Sciences, Australian Centre for Evolutionary Biology and Biodiversity, The University of Adelaide, South Australia, Australia*

Phone +61 8 8303 5148

Fax +61 8 8303 4364

Email [phyllis.macgillivray@adelaide.edu.au](mailto:phyllis.macgillivray@adelaide.edu.au)

Irene L. Hudson

*School of Mathematical and Physical Sciences, University of Newcastle, Callaghan, NSW, Australia*

John G. Conran

*School of Earth and Environmental Sciences, Australian Centre for Evolutionary Biology and Biodiversity, The University of Adelaide, South Australia, Australia*

Andrew J. Lowe

*School of Earth and Environmental Sciences, Australian Centre for Evolutionary Biology and Biodiversity, The University of Adelaide, South Australia, Australia and State Herbarium of South Australia, Science Resource Centre, Department of Environment and Natural Resources, South Australia, Australia.*



# Statement of Authorship

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Publication Status	<input checked="" type="checkbox"/> Published <input type="checkbox"/> Accepted for Publication <input type="checkbox"/> Submitted for Publication <input type="checkbox"/> Publication Style
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## Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Phyllis (Fran) MacGillivray		
Contribution to the Paper	Sought and obtained funding; interpreted all analyses; wrote manuscript as principal author; named as corresponding author		
Signature		Date	26 Oct 2012

Name of Co-Author	Irene L Hudson		
Contribution to the Paper	Performed all GAMLSS analysis; helped to evaluate and edit the manuscript		
Signature		Date	19 October 2012

Name of Co-Author	John G Conran		
Contribution to the Paper	Advised on <i>Diuris</i> taxonomy and field sites; helped to evaluate the manuscript		
Signature		Date	24 Oct 2012

Name of Co-Author	Andrew J Lowe		
Contribution to the Paper	As principal supervisor, advised on development of the manuscript.		
Signature		Date	23.10.12



## **Abstract**

This investigation of the climatic drivers associated with long-term trends in the flowering phenology of three species of *Diuris* (Donkey) orchids is based on a 110-year data set sourced from Australia's Virtual Herbarium from southern Australia. Generalized Additive Models for Location, Scale and Shape (GAMLSS) incorporated main and interactive effects of year, season, species, temperature, rainfall, longitude, latitude and altitude as predictors of peak flowering day. A highly significant flowering response to mean minimum seasonal temperature and seasonal rainfall was identified with shifts to earlier flowering in warmer and drier seasons expected under climate change scenarios ( $\sim 3.5$  days  $^{\circ}\text{C}^{-1}$ ;  $\sim 2.2$  days  $100\text{ mm}^{-1}$  seasonal rain). Earliest flowering thresholds occurred with minimum temperatures of around  $9^{\circ}\text{C}$  and rainfalls between 75 mm and 125 mm.

Overall a trend towards earlier flowering was confirmed which approximates the pattern of increase in global average sea-surface temperatures over the same period. GAMLSS change point years 1932/33 and 1973/74 indicate two distinct periods during which flowering in the central and western regions advanced at a high rate, separated by a period in the middle of the century when flowering time advanced more slowly. This was a period during which sea-surface temperatures fell, the Inter-decadal Pacific Oscillation showed a significant decline and La Niña phases predominated. In eastern regions, flowering was delayed post 1932. The impact of climate change on the flowering phenology of these species is expected to result in greater temporal overlap among species increasing the potential for reproductive success through greater opportunities for pollination and hybridization. This analysis demonstrates the suitability of GAMLSS for extracting information and improving predictions related to processes obscured by synergistic interactions, and for differentiating local from broad regional climatic impacts, and further demonstrates the value of herbarium data sets and their capacity to determine changing synchronies between interacting species at a given location under ongoing climate change.

**Keywords:** *Flowering phenology; Climate change; Herbarium records; Diuris; General Additive Models for Location, Scale and Shape (GAMLSS); latitude, altitude*

**Abbreviations:** BOM Bureau of Meteorology; GAMLSS General Additive Models for Location Scale and Shape; PRM Primary Reference Model; SM1 Support Model 1; SM2 Support Model 2; PFD peak flowering day; ENSO El Niño Southern Oscillation; IPO Inter-decadal Pacific Oscillation

## Introduction

For plants with animal-mediated pollination syndromes (Ramírez, Eltz et al. 2011), reproductive success necessitates the close coincidence of flowering time with the emergence of pollinating agents (Bascompte and Jordano 2007; Tylianakis, Didham et al. 2008; Yang and Rudolf 2010). One of the major concerns over contemporary anthropogenically-forced climate change is the extent to which these time-sensitive relationships may be disrupted by differential phenological shifts resulting in temporal mismatches (Parmesan 2007; Miller-Rushing, Høye et al. 2010). The intimate relationship which exists between many orchids and their pollinating insects provides a perfect example. Indeed, it was Charles Darwin (Darwin 1862) who first grasped the evolutionary implications of the remarkable adaptations of orchid floral morphologies for fertilization by their specific insect pollinators.

While compelling evidence now links phenological shifts to a changing climate (Menzel, Sparks et al. 2006; Rosenzweig, Karoly et al. 2008), much of our current knowledge about the nature of this response to prevailing local conditions derives from observations across the mid to higher latitudes of the northern hemisphere where the main regulator of flowering is a response to winter accumulated heat sums (Snyder, Spano et al. 2001; Clark and Thompson 2010; Ohashi, Kawakami et al. 2011). However, in Mediterranean-type climates, the impacts of high summer temperatures and unreliable rainfall have been

shown to have a greater regulatory influence (Peñuelas, Filella et al. 2004; Tylianakis, Didham et al. 2008).

Recent studies are revealing the nature of the response of Australian flora to climatic change (Hudson and Keatley 2010a). An investigation of *Diuris* orchid phenology in South Australia from 1897 to 2005 found an overall shift to earlier flowering of  $-0.17$  days  $\text{year}^{-1}$  (MacGillivray, Hudson et al. 2010), and a study of shifts in first flowering dates revealed a response to the general warming experienced in south-eastern Australia during the period 1983-2006, by 38 out of 65 plant species, mostly herbs (Keatley and Hudson 2007b). A mean advancement of  $0.9$  days  $\text{year}^{-1}$  was reported, with eight perennials showing a significant shift to earlier flowering and five to later. These shifts were recently confirmed (Keatley and Hudson 2012) and shown to be significantly associated with temperature and rainfall, both separately and in combination (Keatley, Hudson et al. 2004; Keatley and Hudson 2008).

Rising global average sea-surface temperatures impact on the Australian climate via the dominating influence of the El Niño Southern Oscillation (ENSO) system, modulated by the Inter-decadal Pacific Oscillation (IPO) (Power, Tseitkin et al. 1999; Chiew and Leahy 2003; Izumo, Vialard et al. 2010; Kirono, Chiew et al. 2010), but see Wolter and Timlin (2011) and the Indian Ocean Dipole (Izumo, Vialard et al. 2010). In concert, these systems substantially influence temperature and rainfall patterns across the continent (Power, Tseitkin et al. 1999; Chiew and Leahy 2003; Murphy and Timbal 2008). In the already drought prone southern coastal areas of South Australia, climate models predict a further reduction in winter (JJA) and spring (SON) rainfall, and increased frequency and variability of drought as the 21<sup>st</sup> century progresses (IPCC 2007) – (Kiem and Franks 2004). A general long-term air-temperature rise which began around 1910 is predicted to continue, increasing by up to  $6^{\circ}\text{C}$  over most of the continent by 2070 (IPCC 2007). This scenario will be exacerbated by an increase in high daily temperature extremes (Christensen, Hewitson et al. 2007) and enhanced evaporation rates, further reducing available moisture.

Herbaria collections have been validated as a suitable source of data able to provide powerful evidence for the historical influence of climate on flowering phenology (Primack, Imbres et al. 2004; Miller-Rushing, Primack et al. 2006; Kelly 2010; Keatley, Hudson et al. 2011; Robbirt, Davy et al. 2011; Zalamea, Munoz et al. 2011; Panchen, Primack et al. 2012). Such data sets often comprise reliable long-term flowering records spanning the full extent of a species' range, and thus offer a source of baseline data by which to gauge and predict future biological and spatio-temporal shifts (Suarez and Tsutsui 2004). In the previous investigation by MacGillivray, Hudson et al. (2010), GAMLSS modelling of an herbarium derived data set showed a significant curvilinear trend with peak flowering of *Diuris orientis* advancing over time and change point years (Abu-Taleb, Alawneh et al. 2007) corresponding to abrupt changes in the El Niño Southern Oscillation (ENSO). The current study uses the same long-term herbarium dataset to investigate the impact of climate on the flowering phenology of South Australian *Diuris* orchid species, with a focus on the specific roles of temperature and rainfall as drivers of flowering shifts, the nature of these shifts across regions and elevations, and their potential impact on reproductive biology and opportunities for hybridization (Fitter and Fitter 2002; Seehausen 2004).

## Materials and Methods

### *Study site and species*

Species of the Australian terrestrial orchid genus *Diuris* employ a deceptive pollination strategy (Ramírez, Eltz et al. 2011; Molnár, Tökölyi et al. 2012) and time their mass flowering to coincide with the beginning of the flowering season of sympatric populations of the Australian pea family, Fabaceae. The three species central to this investigation, *Diuris behrii* Schldl., *D. orientis* D.L.Jones, and *D. pardina* Lindl., were chosen on the basis of their wide distribution, ease of recognition and identification, and a short, well defined flowering period with intraspecific synchronicity of flowering at the population level. They are widely distributed throughout much of southern South Australia, growing in heathland, woodland or open eucalypt forest, where all three species can often be found growing in close proximity and flowering synchronously (Supplementary material S1).



Other than during their flowering phenophase, *Diuris* plants are very difficult to detect in the field, and vouchered specimens can generally be relied upon to capture flowering near peak.

Climatic variables were obtained from the Australian Bureau of Meteorology (BOM). Temperature and rainfall records spanning the period covered by the phenological data (1893-2009) were not available from any one station. Data were therefore obtained from two of the most central locations: temperature from the Adelaide (Kent Town) station # 23090 and rainfall data from the station at Glen Osmond # 23005, situated approximately 5km south of Kent Town. Mean minimum winter temperatures increased by  $>1^{\circ}\text{C}$  and spring minima by  $\sim 1.7^{\circ}\text{C}$  over the period represented by the data. Annual rainfall ranged from 304 mm-1010 mm, and although there was little change in mean rainfall over time, falls occurred with much less predictability as the century progressed (see Climate Change p6).

#### ***Data set***

Data relating to 377 documented *Diuris* specimens were sourced from Australia's Virtual Herbarium electronic database. Elimination of incomplete entries left 305 records suitable for analysis, dating from 1893 to 2005. The range of the specimens was bounded by latitudes 38 0S and 32.7 0S from south to north, by longitudes 134.2 0E to 1410E from west to east, and at elevations from sea level to 767 meters. This range is restricted to areas receiving comparatively consistent and reliable rainfall as set down by the BOM Index of Variability (an index for assessing the variability of annual rainfall:

Variability Index = [(90th percentile - 10th percentile) / 50th percentile]  
<http://www.bom.gov.au/climate/change/aus>. Rainfall is more variable at northern latitudes than further south.

Flowering dates, ranging from 31 July to the 16 November, were re-calculated for analysis as the number of days following the winter solstice (June 22=day 1). This was chosen as

an appropriate seasonal event, marking the beginning of the lengthening photoperiod. Climatic data for South Australia covering the same time span as the herbarium data were obtained from the Bureau of Meteorology <http://www.bom.gov.au/climate/change/>. An assumption was made that herbarium specimens are generally best representative of the period around peak flowering (Miller-Rushing, Primack et al. 2006; Robbirt, Davy et al. 2011), although specimens collected in the winter are more likely to represent an early flowering stage. Winter (JJA) flowering of these *Diuris* specimens was restricted to the month of August, with the exception of the very earliest first flowering record on 31 July.

### *Analysis*

Linear regression (Dose and Menzel 2004; Hudson, Keatley et al. 2005; Sparks and Tryjanowski 2005; 2010) has been the major method of analysis used to detect changes in phenological time series (Roberts 2011). Traditionally, phenological dates (first flowering or peak flowering) are plotted against time in years (Bradley, Leopold et al. 1999; Beaubien and Freeland 2000; Abu-Asab, Peterson et al. 2001; Peñuelas, Filella et al. 2002a; Zheng, Ge et al. 2006; Abu-Taleb, Alawneh et al. 2007; Miller-Rushing and Primack 2008; Doi, Takahashi et al. 2010) with the resultant slope of the regression line indicating the mean rate of change of the phenophase given as days per year (MacGillivray, Hudson et al. 2010; Keatley and Hudson 2012). These slopes are frequently the basis of meta-analysis studies to detect phenological changes in response to climate change (Root, Price et al. 2003; Parmesan 2006; Hudson 2010b; 2011). Major disadvantages of this least squares approach are its restriction to time series with a comparatively linear trend, unreliable extrapolation properties, and sensitivity to outliers and boundary values (MacGillivray, Hudson et al. 2010). Only rarely have other curve fitting methods (Ahas 1999; Sagarin and Micheli 2001) been used to detect change (Roberts 2008; Hudson 2010a).

Generalized Additive Models for Location, Scale and Shape (GAMLSS) (Stasinopoulos and Rigby 2007) [URL http://www.R-project.org](http://www.R-project.org) were used here as a curve fitting modelling tool to identify the main climatic drivers of change in flowering phenology from a range of climatic predictors (mean minimum monthly and seasonal temperatures,

and monthly and seasonal rainfall) and environmental parameters (latitude, longitude and elevation) (Supplementary material S2). The benefits of GAMLSS for phenological and herbarium data are that they (i) can identify the main drivers of the event of interest from a multiplicity of predictors, (ii) allow for non-linear impacts of time and/or the explanatory variables and (iii) can statistically detect thresholds such as the lowest temperature for the commencement of flowering (Hudson, Kim et al. 2009; Hudson, Kim et al. 2010). The GAMLSS framework is implemented in a series of packages in R (R Development Core Team 2007), a free software (see [URL http://www.R-project.org](http://www.R-project.org)). The GAMLSS procedure was used with a cubic spline smoothing function (Stasinopoulos and Rigby 2007).

Twelve initial exploratory models of increasing complexity were built by adding together combinations of Year, Species, Season, Altitude, Longitude, Latitude and four climatic parameters, mean minimum temperature and rainfall (both monthly and seasonal), as predictors of flowering time. All models were run in two versions, addressing main effects both separately and in combination with two-way interaction effects. Goodness of fit was assessed by the Akaike Information Criterion (AIC) (Akaike 1983), and the diagnostic plots of residuals and fitted values were used to check for systematic bias or lack of fit in the main effects component of the models (further information provided in Supplementary material S2).

Each model assumes that the flowering series represents normally distributed data and the RS algorithm, a generalization of the algorithm of Rigby and Stasinopoulos (Rigby and Stasinopoulos 1996) was used to obtain the estimates of the predictor effects. Each term in a given model shows the effect of a given predictor, after adjusting for the effects of all other predictors in the model. Interaction plots are only available when at least one of the terms is categorical, and not for interactions between two continuous covariates e.g. temperature and rainfall. Cubic splines allow for non-linearity in the flowering response to year, altitude and the climatic predictors, and diagnostic plots of residuals and fitted values were used to check for systematic bias or lack of fit in the main effects component of the models.

As the flowering period investigated included the coldest months of the year (the winter months June, July, August), and because minimum temperatures are reported to be rising at a greater rate than maximum temperatures (Power, Tseitkin et al. 1998; Janes, Steane et al. 2010), mean minimum air temperatures were considered the most appropriate predictors (Luedeling, Gebauer et al. 2009; Tanino, Kalcsits et al. 2010; Panchen, Primack et al. 2012) and calculated for each monthly and seasonal period under consideration. Rainfall was calculated as the total precipitation for each period. The data were also explored in relation to three latitudinal ( $<34.5^{\circ}$  S;  $34.5^{\circ}$  S -  $35.5^{\circ}$  S;  $>35.5^{\circ}$  S), and three longitudinal regions ( $<138^{\circ}$  E;  $138^{\circ}$  E -  $139^{\circ}$  E;  $>139^{\circ}$  E) and also the three individual species. With the exception of the north-eastern sector, all regional divisions were represented.

## Results

The 12 main effects versions of the models are set out in Supplementary material S2. The principal reference model (PRM) (Model 10) on which the main interpretation of this analysis is based incorporates season of flowering, seasonal climatic variables, year, species, altitude, and the regional variables longitude and latitude (Table 1). GAMLSS term plots of the significant main effects in the principal reference model (PRM) are shown in Fig. 1 with estimates and significance of all terms given in Table 1. For combined species, PFD advanced on average by  $\sim 3.5$  days  $^{\circ}\text{C}^{-1}$  rise in seasonal minima ( $P=0.002$ ) and by  $\sim 2$  days  $100\text{ mm}^{-1}$  decrease in seasonal rainfall ( $P=0.068$ ). Species, season, and seasonal climate were significant as main effects, with altitude significant at 10% ( $P=0.06$ ). Support model, SM1 (Model 11), identical to PRM but with the additional inclusion of monthly climatic variables (Table 1), provides support for PRM estimates. An additional support model, SM2 (Model 4), is similar to the PRM but without regional terms (Table 1). Comparisons with this model serve to highlight the importance of spatial considerations.

***Temporal effects***

Year was not significant as a main effect in the three models under consideration, but seasonal climate acts as a proxy for year, and change over time can be accounted for by change in the seasonal climate over the corresponding period. Negative estimates of -0.112;  $P=0.005$  (PRM) and -0.241;  $P=0.000$  (SM2) for seasonal temperature imply pronounced shifts to earlier flowering have occurred during warmer periods (Table 2). The overall trend approximates the pattern of increase in global average sea-surface temperatures over the same period (Supplementary material S3). Figures 2 and 3 indicate a flowering progression beginning in the south-east of the state and advancing in a north-westerly direction, while differential regional shifts have restricted the overall flowering season over time.

**Table 1.** Estimates and significance of main effects of version 1 (without interactions) of the principal reference model (PRM), support model 1 (SM1) and support model 2 (SM2); \* cubic spline; GD: Global Deviance; AIC: Akaike Information Criterion; SBC: Schwartz Bayesian Criterion.

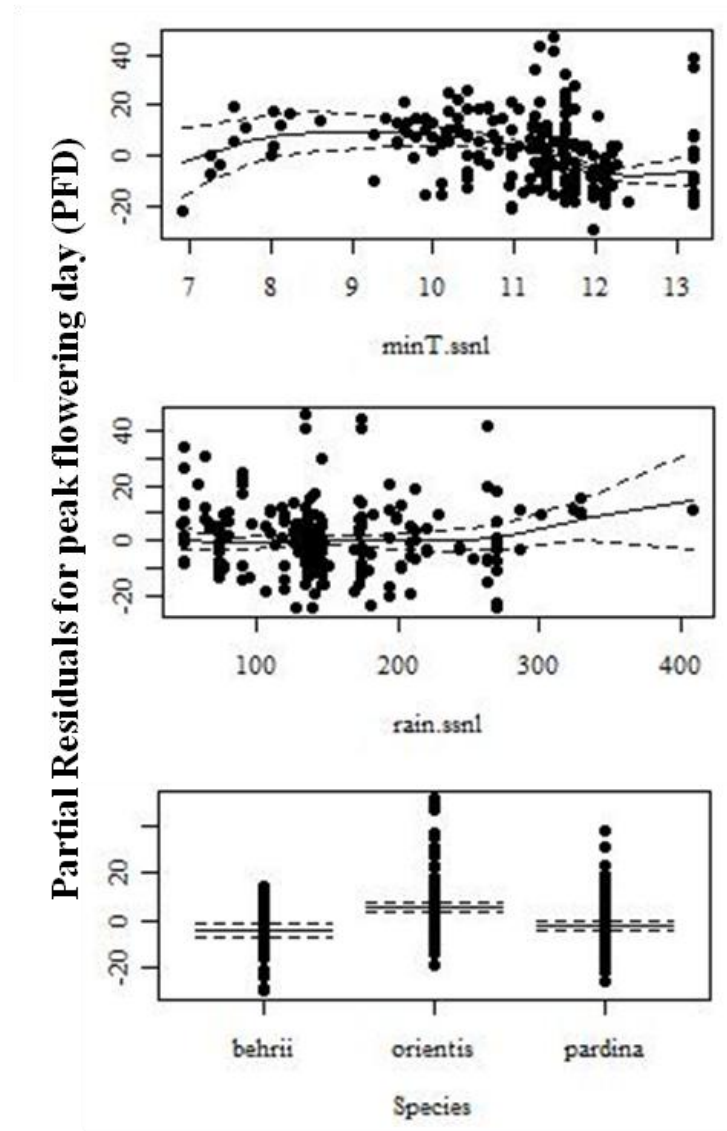
	PRM		SM1		SM2	
	Estimate	Pr(> t )	Estimate	Pr(> t )	Estimate	Pr(> t )
Intercept	1.95E+02	0.008	1.76E+02	<0.001	1.93E+02	0.006
Year*	-3.54E-02	0.382	-3.001E-02	0.299	-3.55E-02	0.370
<i>D. orientis</i> vs. <i>D. behrii</i>	9.92E+00	<0.000	4.40E+00	0.006	9.42E+00	<0.000
<i>D. pardina</i> vs. <i>D. behrii</i>	2.16E+00	0.312	1.66E+00	0.265	2.47E+00	0.251
Season (winter vs. spring)	-3.59E+01	<<0.000	-4.13E+01	<<0.000	-3.65E+01	<<0.000
Altitude*	1.257E-02	0.061	-1.6E-03	0.733	5.85E-03	0.213
Temperature* (monthly)			6.78E+00	<<0.000		
Rain* (monthly)			-1.41E-01	<<0.000		
Temperature* (seasonal)	-3.55E+00	0.002	-8.08E+00	<<0.000	-3.33E+00	0.003
Rainfall* (seasonal)	2.24E-02	0.068	4.62E-02	<0.000	1.92E-02	0.109
Longitude (central vs. west)	-9.03E-01	0.720	1.49E+00	0.397		
Longitude (east vs. west)	2.83E+00	0.252	4.40E+00	0.011		
Latitude (central vs. south)	-2.46E+00	0.266	-7.01E-01	0.668		
Latitude (north vs. south)	-7.44E-01	0.790	9.88E-01	0.628		
GD::	2241.437		2027.366		2246.221	
AIC:	2291.434		2093.360		2288.219	
SBC:	2383.177		2214.457		2365.281	

**Table 2.** Estimates and significance of all significant interaction effects for the interaction versions of the principal reference model (PRM), support model 1 (SM1) and support model 2 (SM2); \* cubic spline; GD: Global Deviance; AIC: Akaike Information Criterion; SBC: Schwartz Bayesian Criterion. (For all main plus interaction effects for the interactive versions of these models see Supplementary material S2)

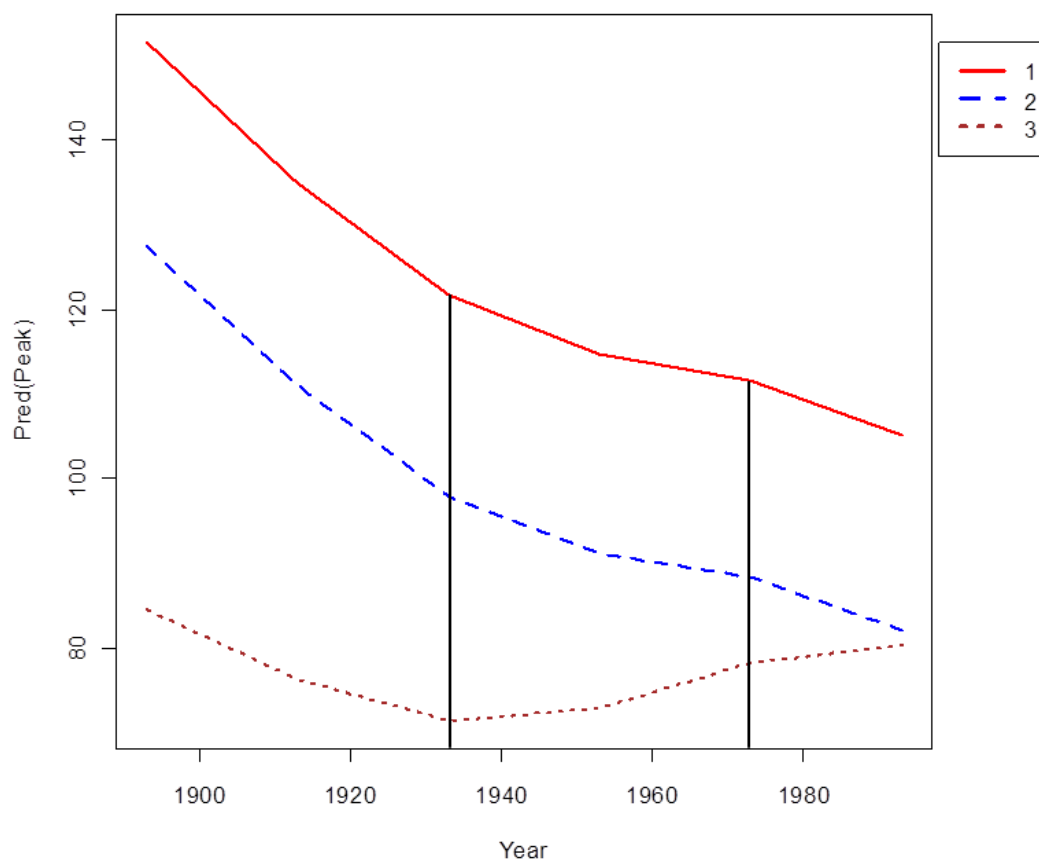
	PRM		SM1		SM2	
	Estimate	Pr(> t )	Estimate	Pr(> t )	Estimate	Pr(> t )
Year*: Season (winter vs. spring)					-0.560	0.055
Year*: Temperature*(seasonal)	-0.112	0.005			-0.241	<<0.000
Year*: Longitude (east vs. west)	0.422	0.019				
Year*: Latitude (north vs. south)			-0.380	0.038		
<i>D. orientis</i> vs. <i>D. behrii</i> : Altitude*	0.052	0.039	0.040	0.017	0.022	0.097
<i>D. pardina</i> vs. <i>D. behrii</i> : Temperature*(monthly)			2.67	0.062		
<i>D. orientis</i> vs. <i>D. behrii</i> : Temperature*(seasonal)			-5.69	0.028		
<i>D. pardina</i> vs. <i>D. behrii</i> : Temperature*(seasonal)			-4.48	0.074		
<i>D. orientis</i> vs. <i>D. behrii</i> : Rain*(seasonal)					0.075	0.055
<i>D. pardina</i> vs. <i>D. behrii</i> : Rain*(seasonal)	0.074	0.099			0.071	0.077
<i>D. orientis</i> vs. <i>D. behrii</i> : Longitude (central vs. west)			-22.3	0.020		
<i>D. pardina</i> vs. <i>D. behrii</i> : Longitude (central vs. west)			-23.2	0.022		
<i>D. pardina</i> vs. <i>D. behrii</i> : Latitude (central vs. south)	-31.1	0.032				
<i>D. pardina</i> vs. <i>D. behrii</i> : Latitude (north vs. south)	-35.9	0.031				
Season (winter vs. spring): Temperature*(seasonal)			32.6	0.010		
Season (winter vs. spring): Latitude (central vs. south)	139	0.069				
Season (winter vs. spring): Latitude (north vs. south)	135	0.068				

Altitude*:Temperature*(seasonal)				0.021	0.007
Altitude*:Rain*(seasonal)				0.000	0.002
Altitude*: Latitude (central vs. south)			0.035	0.068	
Altitude*: Latitude (north vs. south)			0.052	0.060	
Temperature*(monthly): Rain*(monthly)			-0.077	0.002	
Temperature*(monthly): Temperature*(seasonal)			-1.94	0.005	
Temperature*(monthly): Rain*(seasonal)			0.057	<<0.000	
Temperature*(monthly): Longitude (central vs. west)			-6.52	<0.001	
Temperature*(monthly): Longitude (east vs. west)			-5.87	0.004	
Temperature*(monthly): Latitude (central vs. south)			3.87	0.071	
Temperature*(monthly): Latitude (north vs. south)			4.16	0.068	
Rain*(monthly): Rainfall*(seasonal)			0.001	0.013	
Temperature*(seasonal): Rain*(seasonal)				0.051	0.019
Temperature*(seasonal): Longitude (central vs. west)	15.9	<0.001	8.74	0.010	
Temperature*(seasonal): Latitude (central vs. south)	11.0	0.025			
Temperature*(seasonal): Latitude (north vs. south)	11.9	0.054			
Longitude (central vs. west): Latitude (central vs. south)			-17.5	0.016	
Longitude (east vs. west): Latitude (central vs. south)	26.3	0.021			
Longitude (central vs. west): Latitude (north vs. south)			-40.8	<0.001	
GD:	2137.515		1828.685		2194.965
AIC:	2289.512		2040.680		2276.962
SBC:	2568.418		2429.677		2427.422

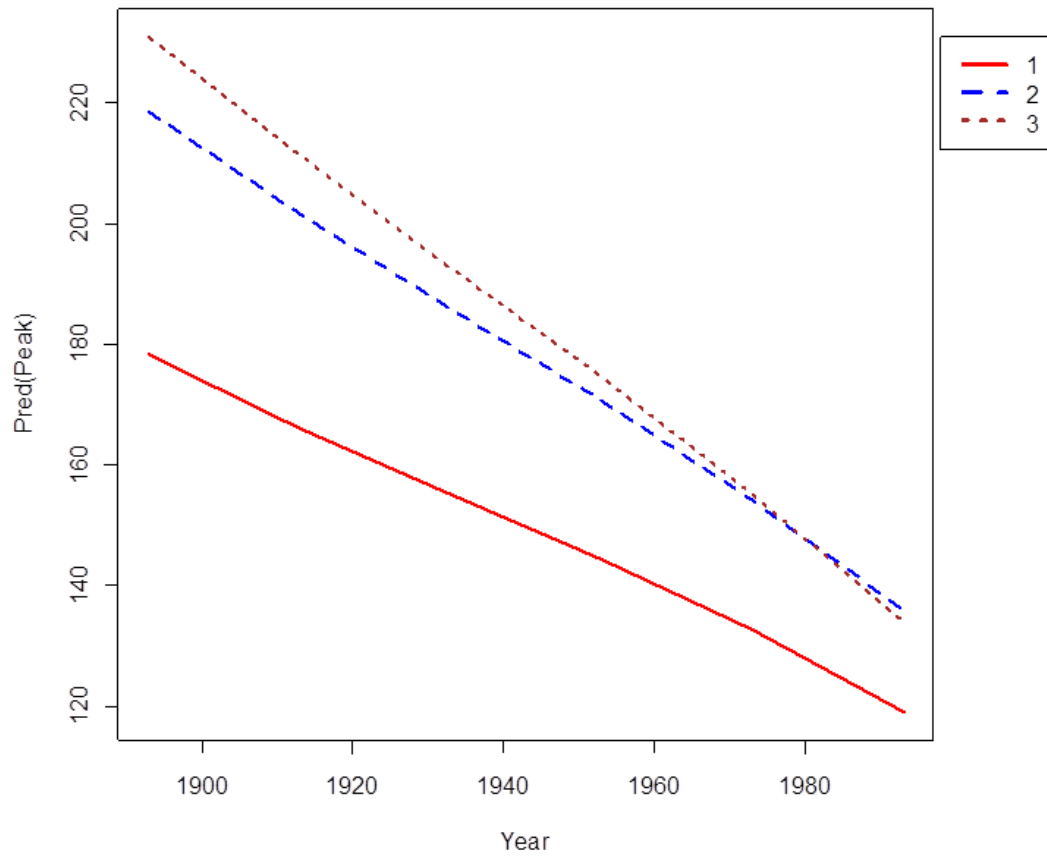




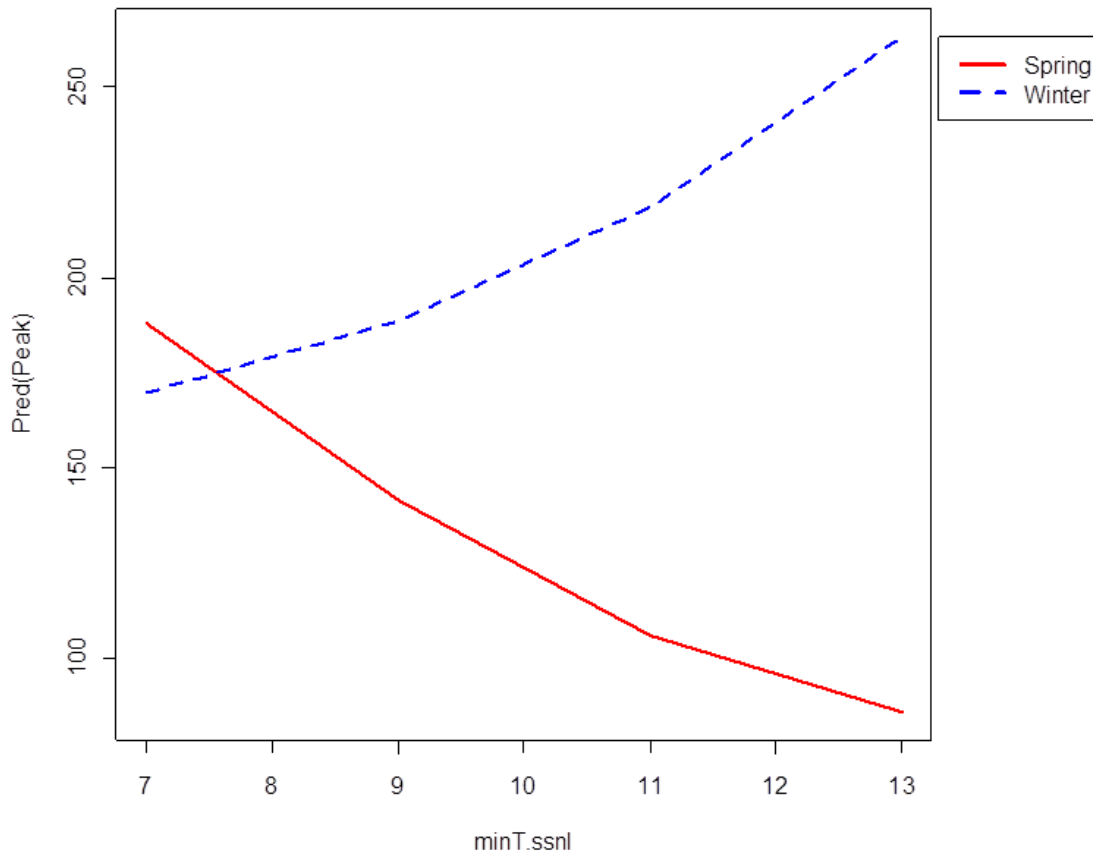
**Fig. 1** Term plots of the significant main effects for mean minimum seasonal temperature (minT.ssnl), seasonal rainfall (rain.ssnl) and species in the principal reference model (PRM). Estimates and significance of all terms are given in Table 1. The GAMLSS spline line and 95% confidence band show the effects on peak flowering date (PFD) for combined species. On average, PFD advanced by  $\sim 3.5$  days  $^{\circ}\text{C}^{-1}$  rise in seasonal minima ( $P=0.002$ ) and by  $\sim 2$  days  $100\text{mm}^{-1}$  decrease in seasonal rainfall ( $P=0.068$ ). Partial residual plots show the relationship between the given independent variable (X axis) and the response variable, peak flowering day (PFD), on the Y axis.



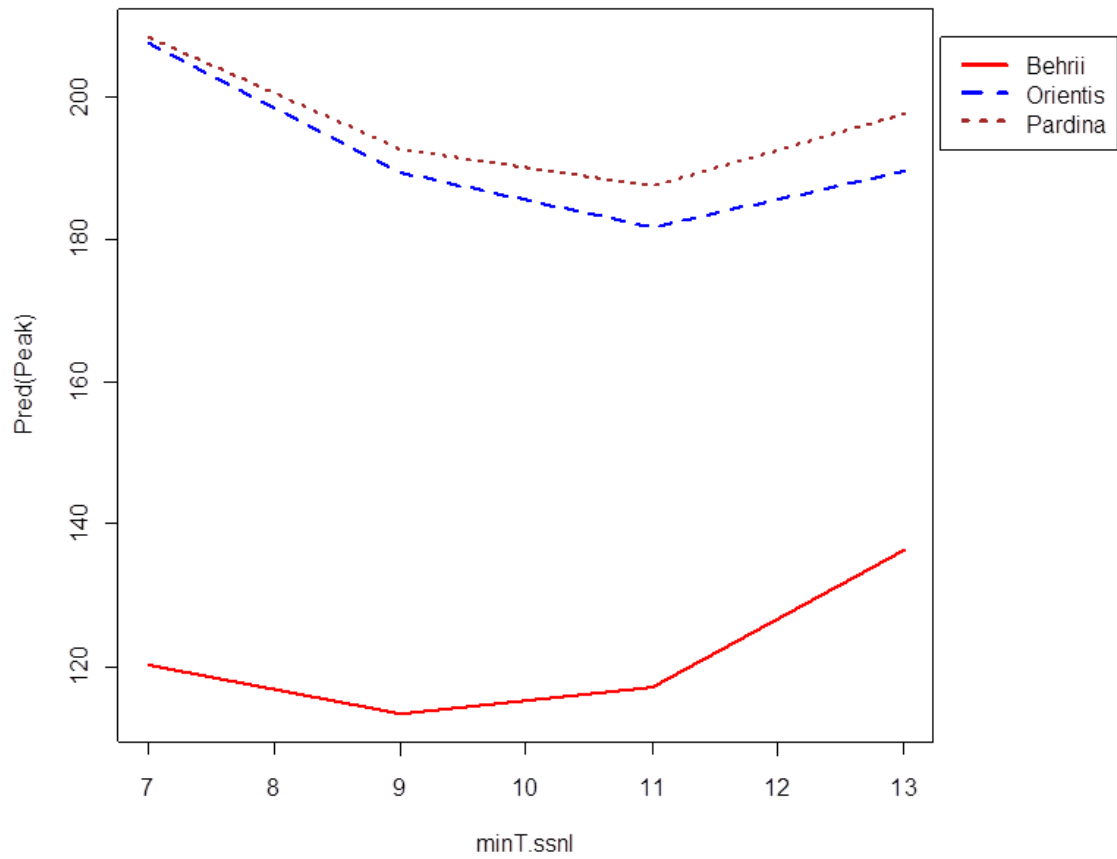
**Fig. 2** Differential year effect across longitudinal regions (PRM) with a significant difference in PFD between western (region 1) and eastern region (3) ( $P=0.019$ ). Shifts over time have been more pronounced in the central and western regions such that differences in flowering time across the range from east to west have diminished as the century progressed. GAMLSS change point years 1932/33 and 1973/74 (vertical lines) identify two distinct periods during which flowering in central and western regions advanced at a high rate, separated by a period in the middle of the century when flowering time advanced more slowly. This was a period during which seas-surface temperatures fell, the IPO showed a significant decline and La Niña phases were predominant (Supplementary material S3). In eastern regions, flowering was delayed post 1932. Y axis labels indicate the predicted peak Pred(Peak) flowering day.



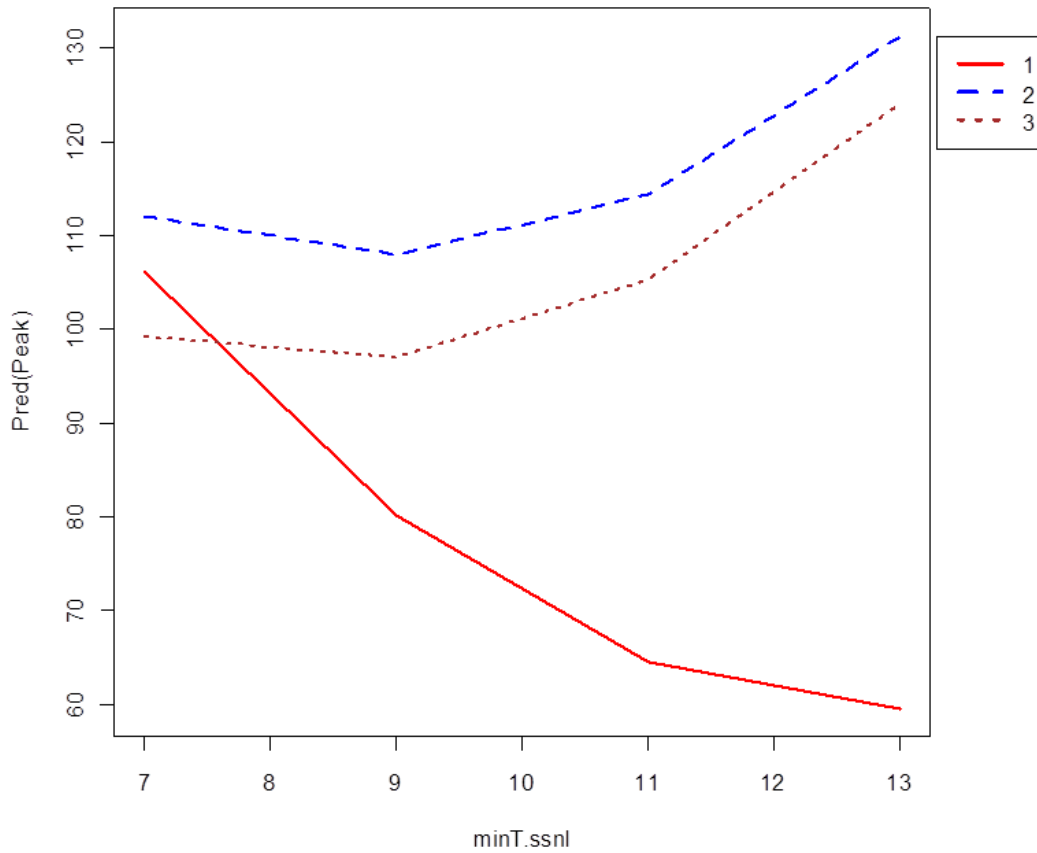
**Fig. 3** Differential year effect across latitudinal regions (SM1) (southern:1; central:2; northern:3). This regional effect is manifest as a more pronounced trend in central and northern regions ( $P=0.038$ , Table 2) with respect to the south. Y axis labels indicate the predicted peak Pred(Peak) flowering day.



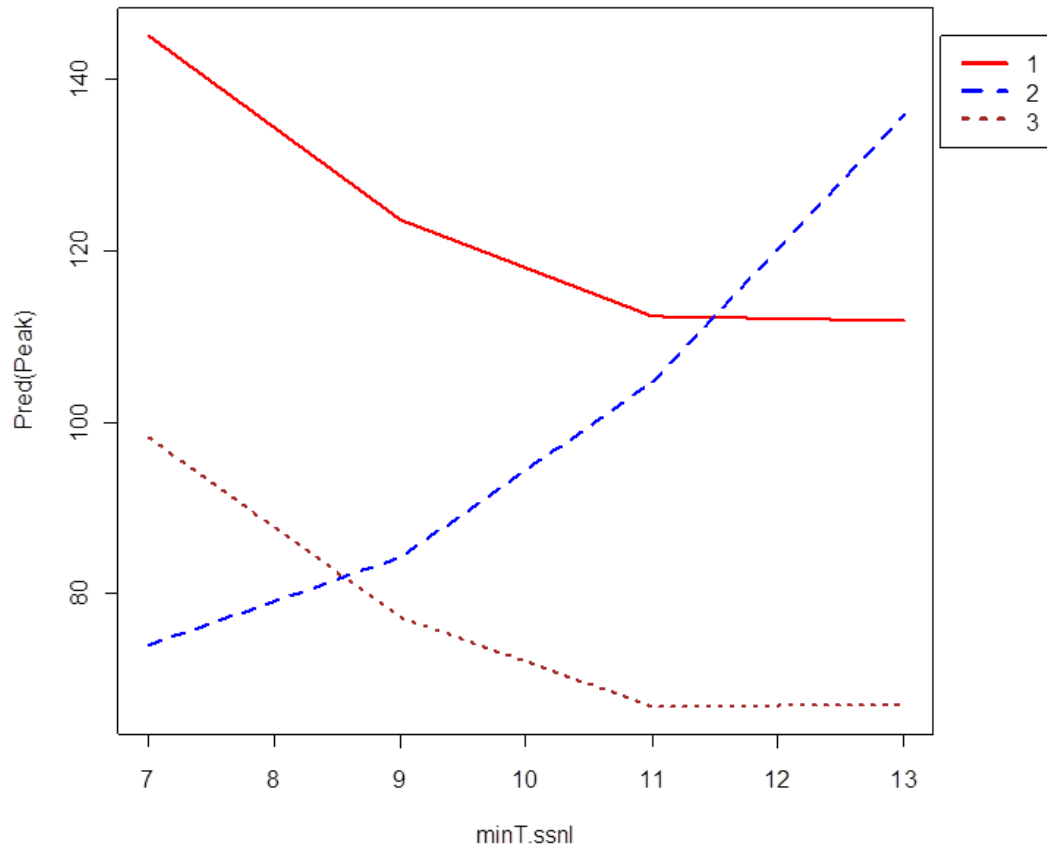
**Fig. 4** Interaction effect of season and mean minimum seasonal temperatures (minT.ssnl) (SM1:  $P=0.0098$ ). Temperature impacts differently across seasons: higher minimum temperatures in winter delay predicted PFD; higher minimum temperatures in spring advance predicted PFD, with an overall shortening of the flowering season. Minimum winter temperatures are generally  $\leq 9^{\circ}\text{C}$ , and winter flowering predictions shown in this plot are highly theoretical in the higher temperature range. Y axis labels indicate the predicted peak Pred(Peak) flowering day; minT.ssnl: minimum seasonal temperature  $^{\circ}\text{C}$ .



**Fig. 5** Differential effect of seasonal temperature (minT.ssnl) across species: *D. behrii* is less responsive to temperature than *D. orientis* (SM1:  $\beta=-5.69$ ;  $P=0.028$ ) and is shown to flower much earlier than the other two species. There is some indication of an early flowering threshold determined by temperature: earliest flowering occurs around 9°C for *D. behrii*, and around 11°C for *D. orientis* and *D. pardina*. Y axis labels indicate the predicted peak Pred(Peak) flowering day; minT.ssnl: minimum seasonal temperature °C.



**Fig. 6** Impacts of temperature differ across the range. With higher seasonal temperatures (minT.ssnl), a substantial shift to earlier flowering in the west (region 1) contrasts with delayed flowering in the central (region 2) and eastern (region 3) longitudinal regions (PRM:  $\beta=15.9$ ;  $P=0.001$ ). While shifts east of longitude  $39^{\circ}\text{E}$  occur in unison, flowering is consistently later in the central region which features the higher altitudes of the Mt Lofty Ranges. Y axis labels indicate the predicted peak Pred(Peak) flowering day; minT.ssnl: minimum seasonal temperature  $^{\circ}\text{C}$ .



**Fig. 7** Impacts of temperature (minT.ssnl) differ across latitudes: a substantial shift to earlier flowering is predicted for southern latitudes (region 1) with respect to central (region 2) (PRM:  $P=0.025$ ) and northern regions (region 3) ( $P=0.055$ ) resulting in an extended flowering season from south to north. Extreme hot and dry conditions may have serious consequences for orchids flowering too late in the season at the northern limits of their range. Y axis labels indicate the predicted peak Pred(Peak) flowering day; minT.ssnl: minimum seasonal temperature °C.

### *Seasonal climatic effects*

As a main effect, mean minimum seasonal temperature is a significant predictor of flowering phenology with an average advance in PFD of between 2.5 - 4.5 days  $^{\circ}\text{C}^{-1}$  increase in temperature ( $P=0.002$ ) (Table 1; Fig. 1). Temperatures around  $9^{\circ}\text{C}$  demarcate winter from spring, and distinguish dissimilar seasonal responses to increasing temperatures (Fig. 4). This plot shows a seasonal temperature effect:  $\beta=32.6$ ;  $P=0.010$ ) (Table 2) by which a considerable advance in PFD during spring in warmer years more than compensates for a delay in winter flowering. There are indications of an early flowering threshold determined by temperature, with earliest flowering occurring around  $9^{\circ}\text{C}$  for *D. behrii*, and around  $11^{\circ}\text{C}$  for *D. orientis* and *D. pardina* (SM1) (Fig. 5).

This seasonal temperature effect is further moderated by a pronounced differential flowering response across regions. Below a minimum of  $9^{\circ}\text{C}$ , earliest flowering occurs in the central longitudinal region; under warmer conditions, a substantial shift to earlier flowering in the west contrasts with delayed flowering in the central (region 2) and eastern (region 3) longitudinal regions (PRM:  $\beta=15.9$ ;  $P=0.001$ , Table 2). While shifts east of longitude  $39^{\circ}\text{E}$  occur in unison, flowering is consistently later in the central region which features the higher altitudes of the Mt Lofty Ranges. Impacts of temperature also vary across latitudes. In warmer seasons, substantial shifts to earlier flowering in the south contrast with delays in central (PRM:  $\beta=11.0$ ;  $P=0.025$ ) and northern regions (PRM:  $\beta=11.9$ ;  $P=0.054$ ) and result in an extended flowering season over the range (Table 2) from south to north (Figures 6 and 7).

Seasonal rainfall had less impact than temperature, but with evidence of a trend to later flowering of around 2 days  $100\text{ mm}^{-1}$  seasonal rainfall ( $\beta=0.022$ ;  $P=0.068$ ) (Table 1; Fig 1). The SM2 plot (Fig. 8) shows a significant differential rainfall effect across species (*D. orientis*  $P=0.055$ ; *D. pardina*  $P=0.077$ ), with *D. behrii* less responsive than the other two species for seasonal falls greater than  $\sim 100\text{ mm}$ . This plot further identifies a lower rainfall threshold for early flowering. Earliest flowering occurs when seasonal precipitation falls between 75mm-125mm; beyond this range, in both wetter and extremely dry seasons, PFD for all species occurs later. Greater temporal overlap of the three species is expected in drier seasons. Without regional considerations, a significant



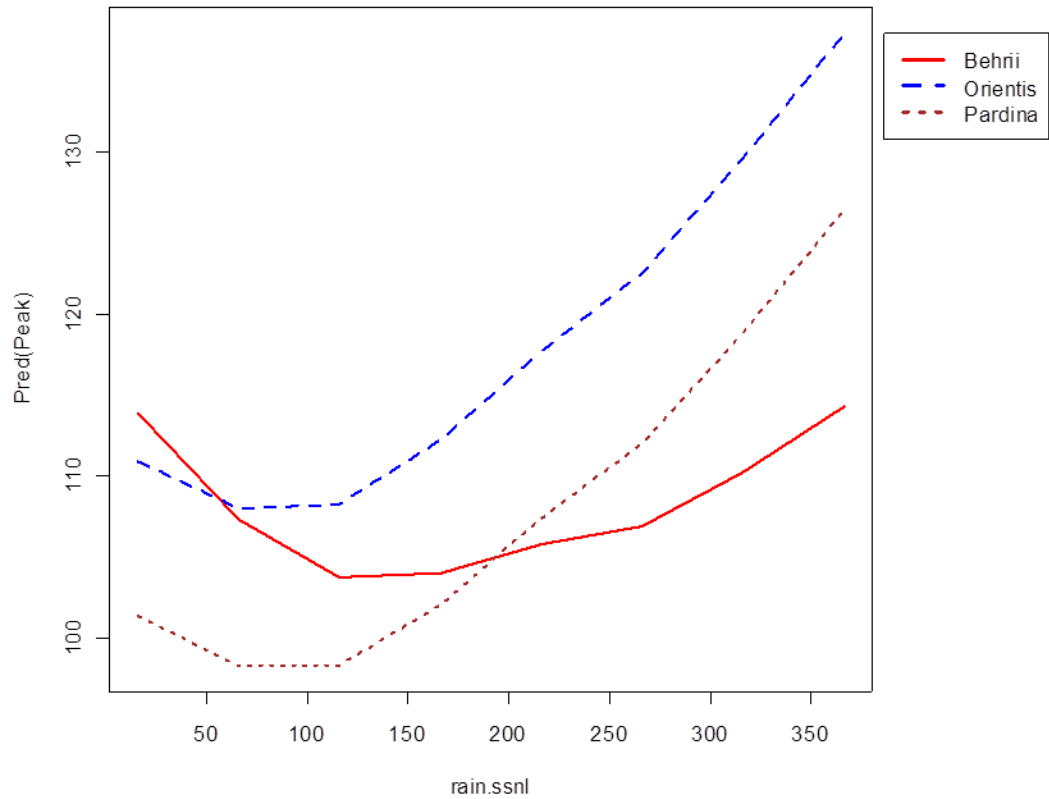
interaction between seasonal temperature and seasonal rainfall (SM2:  $\beta=0.051$ ;  $P=0.019$ ) implies an earlier flowering response in warmer, drier conditions (Table 2).

### *Differential species effects*

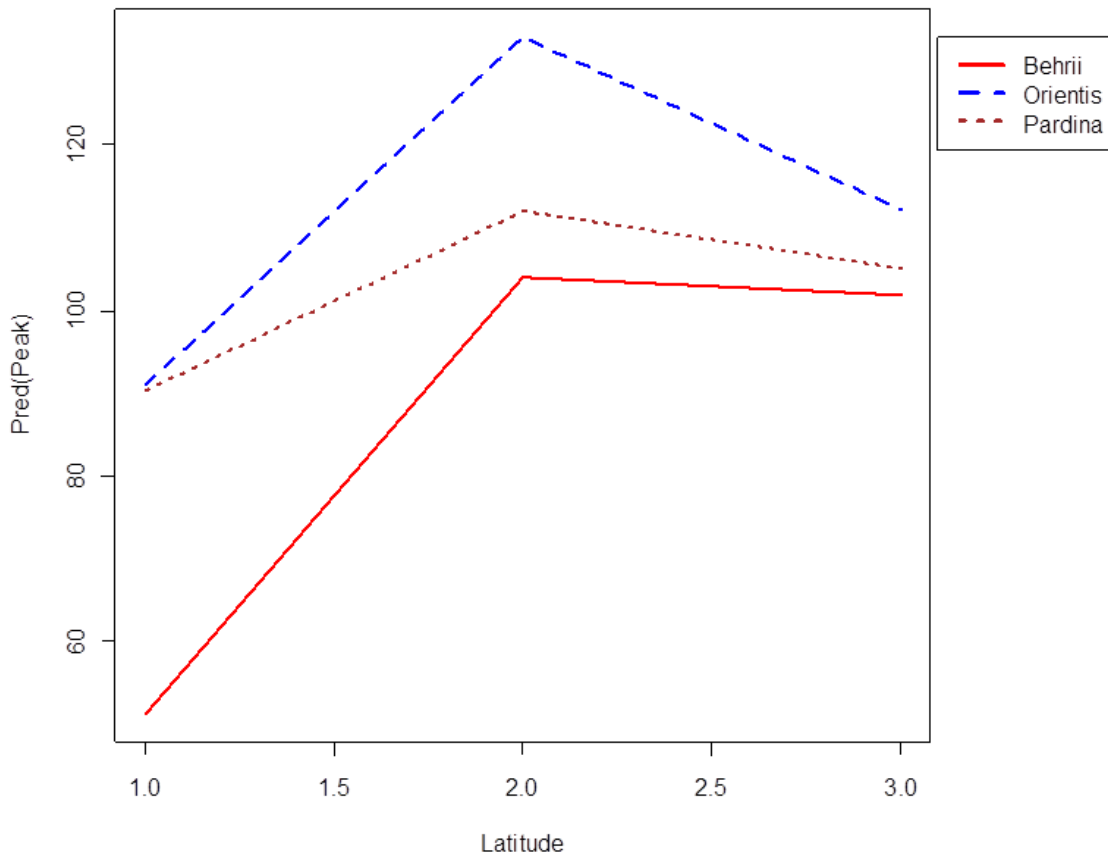
Species differences are consistently present and significant as a main effect in the three models (Table 1). Generally, *D. behrii* is the earliest of the three species, reaching peak flowering on average about nine days before *D. orientis* ( $P<0.001$ ) (Fig. 1). *D. behrii* is less responsive than *D. orientis* to temperature: (SM1: ( $\beta=-0.69$ ;  $P=0.028$ ) (Fig. 5), and less responsive than the other two species to rainfall (SM2: *D. orientis*  $\beta=0.075$ ;  $P=0.055$ ; *D. pardina*  $\beta=0.071$ ;  $P=0.077$ ) (Fig. 8; Table 2). In wet seasons, *D. behrii* is the earliest flowering, in very dry seasons the order is reversed. These relative shifts result in greater temporal overlap of the three species in warmer and drier seasons.

Peak flowering of all species occurs first in the south, but with *D. behrii* well ahead of the other two species. At central latitudes, delayed flowering of *D. behrii* with respect to *D. pardina* (PRM:  $\beta=-31.1$ ;  $P=0.032$ ) lessens the temporal gap, and in the north all species flower within a similar time-frame (PRM:  $\beta=-35.9$ ;  $P=0.031$ ) (Fig. 9; Table 2). Altitude was significant only at 10% (PRM). Delayed flowering at higher altitudes with respect to flowering down slope ( $\beta=+0.013$ ;  $P=0.061$ ) (Table 1) is due to delays at higher elevations for *D. orientis* with respect to *D. behrii* (and *D. pardina*) ( $\beta=+0.052$ ;  $P=0.039$ ) (Table 2, Fig.10). An opposing trend to earlier flowering of the latter two species serves to increase divergence in flowering times along elevation gradients. This trend was supported by my field observations of *D. orientis* flowering later by 10 days 100 meters<sup>-1</sup> ( $R^2=0.54$ ;  $P=0.000$ ) in Belair National Park in 2009.

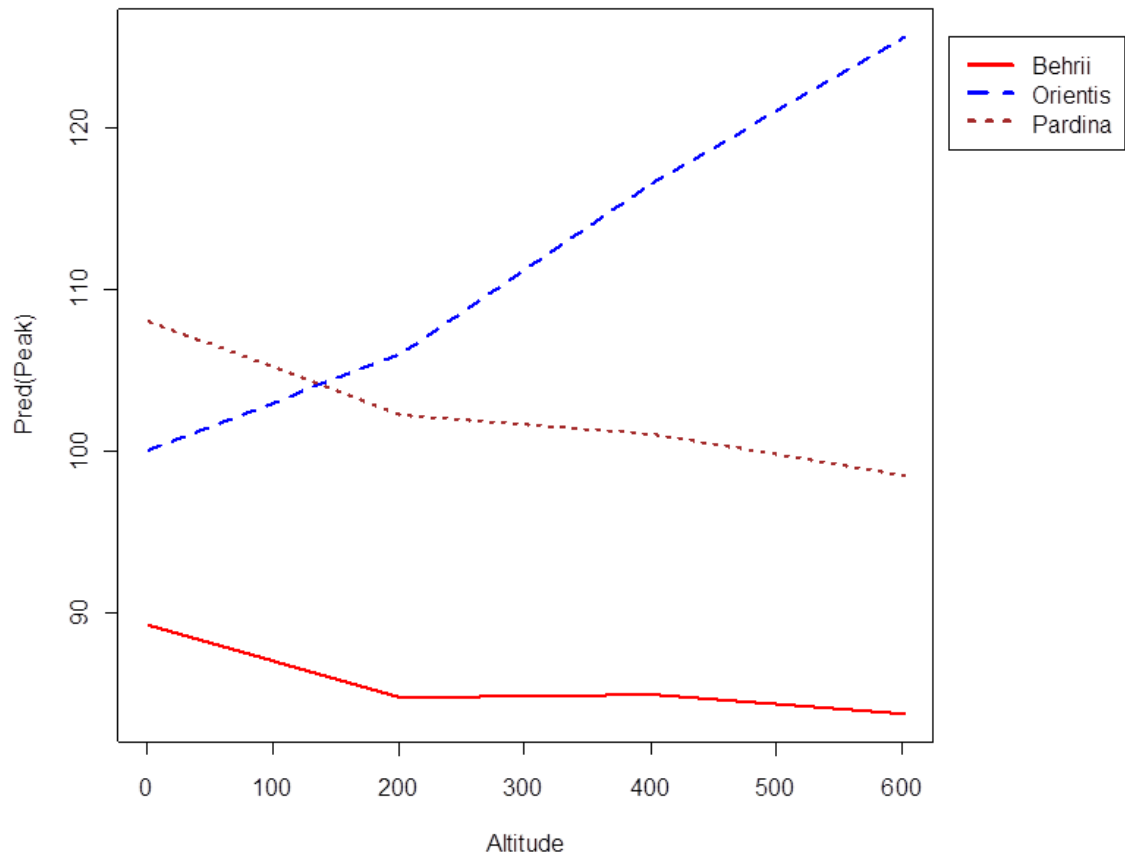




**Fig. 8** Differential effect of seasonal rain (rain.ssnl) across species (SM2: *D. orientis*  $P=0.055$ ; *D. pardina*  $P=0.077$ ). With seasonal rainfalls greater than ~100 mm, *D. behrii* is less responsive than the other two species. This plot further identifies a lower rainfall threshold for early flowering: earliest flowering of all species occurs with falls from ~ 75mm and 125mm, below which flowering events are delayed. Greater temporal overlap of the three species expected in drier seasons. Y axis labels indicate the predicted peak Pred(Peak) flowering day; rain.ssnl: seasonal rainfall (mm).



**Fig. 9** The extent of temporal overlap depends on location due to differential species effects from south (region 1) to north (region 3) with significant differences between *D. behrii* and *D. pardina* PRM;  $P=0.03$ . All species flower earlier in the south, but with *D. behrii* significantly earlier than *D. pardina* and *D. orientis*; in the north all species flower within a similar time-frame. Y axis labels indicate the predicted peak Pred(Peak) flowering day. Latitudinal regions 1.5 and 2.5 on X axis have no meaning in this context.



**Fig. 10** A significant differential species effect with altitude (PRM;  $P=0.039$ ) with large delays for *D. orientis* with respect to *D. behrii* (and *D. pardina*) at higher elevations. There is evidence of an opposing trend to earlier flowering of the latter two species, increasing divergence in flowering time between *D. orientis* and the other two species along an elevation gradient. Y axis labels indicate the predicted peak Pred(Peak) flowering day. Altitude in meters.

## Discussion

This analysis provides consistent evidence that the flowering phenology of *Diuris* orchids is highly responsive to both temperature and rainfall, with a shift to earlier flowering under warmer and drier conditions during the 112 year period. Mean minimum seasonal temperature was a significant predictor of flowering phenology with an average advance in peak flowering of around three and a half days for each degree centigrade rise in temperature. This overall advance obscures opposing seasonal responses whereby a substantial advance in temperature during spring over compensates for a delay in winter flowering; an effect which results in a contraction of the flowering season, also reported by (Yu, Luedeling et al. 2010). Delayed flowering in warmer winters may be due to the longer pre-flowering period needed to compensate for vernalization processes which are inhibited above requisite low temperatures (Cave and Johnston 2010), resulting in an earliest flowering threshold with temperatures of around 9°C.

Seasonal rainfall did not have the predictive power of temperature, although all models provide consistent evidence of a trend to advanced flowering in drier seasons. The lack of a clear rainfall signal may be partly due to the change in rainfall patterns from south to north. Whereas rainfall in southern regions is relatively reliable and consistent, both throughout the season and from year to year, at northern range limits variable and unpredictable events are typical. A early flowering threshold was again identified, with delayed flowering in extremely dry seasons. This phenomenon will most likely have greatest impact at the northern range limits, where delayed flowering under dry conditions will increase exposure to, and heighten the effect of, extreme heat events. Rainfall also impacts on synchronicity, with a contraction of the flowering season and consequential increase in temporal overlap of the three species in drier years.

Important geographical influences were revealed by comparisons of estimates obtained in the PRM model with regional terms, and model SM2 without. In a region where temperature and rainfall patterns vary considerably from north to south, and a mountain range separates east from west, it is not surprising that changes are dependent on location (Borchert 1996; Keatley, Hudson et al. 2011). In the cooler and wetter south-east,

pronounced shifts to earlier flowering were opposed to delays further north, with possible serious consequences for northern populations where limiting soil moisture in association with high maximum temperatures are likely to have deleterious impacts on late flowering events. The delayed flowering of *D. orientis* at higher altitudes with respect to populations down slope may account for the disparity in flowering times in the central longitudinal regions. Few other altitudinal effects have been clearly identified, but this important geographical effect is deserving of further investigation, as many remnant populations occur along the highly populated ranges, where severe restrictions imposed by loss of habitat increase their vulnerability to the potentially disruptive effects of climate change.

This investigation has also revealed additional species sensitivities which may impact on long-term population vulnerability. The earliest flowering and least abundant of the three species, *D. behrii*, is the least responsive to changes in the prevailing climate; the latest flowering, *D. orientis*, is the most responsive and the most abundant. Under conditions of projected change, this differential species effect is predicted to increase phenological overlap. Also, as warming induces earlier flowering, the later flowering species have much more ‘room to move’ than the earlier flowering *D. behrii*. A survival advantage for species with greater phenological plasticity (Bradley, Leopold et al. 1999; Willis, Ruhfel et al. 2008; Primack and Miller-Rushing 2012), implies that *D. behrii*, presently the most vulnerable of the three species, may be under greatest threat in the face of ongoing change. The extent of the overall flowering season is currently more restricted than it was when collecting began, but greater temporal overlap among species along with greater opportunities for pollination and hybridization will vary with location and species.

## Conclusions

The overall trend to earlier flowering approximates the pattern of increase in global average sea-surface temperatures over the corresponding period. GAMLSS change point years 1932/33 and 1973/74 distinguish two distinct episodes of rapid flowering advancement, separated by a period in the middle of the century when flowering advanced little, and was even delayed in the eastern part of the range. This coincided with falling sea-surface temperatures, a significant decline in the IPO and dominating La Niña. Under

dominating global weather systems moderated by regional geographic influences, the impact of climate change on the flowering phenology of these species is expected to manifest most profoundly at the population level. Populations at the northernmost limits of the range, flowering later during years of drought, may be further threatened by extreme heat, and *D. behrii*, the least responsive and most vulnerable of the species, is in danger of further decline (Willis, Ruhfel et al. 2008). For these food deceptive orchids, corresponding phenological shifts of Fabaceae species will also be influential on long-term reproductive success.

The statistically robust GAMLSS modelling of peak flowering day effectively identified a significant, species specific, flowering response to mean minimum seasonal temperature and seasonal rainfall with non-linear regional trends over time. Shifts to earlier flowering over time at lower, as opposed to higher, latitudes are contrary to those reported for the northern hemisphere (Parmesan 2007; Primack, Ibáñez et al. 2009). As drivers of ecological and evolutionary change, interactions between temperature and rainfall may have greater impact than either factor acting alone (Gotelli and Ellison 2004; Nicholls 2004; Hudson 2010a; Kelly 2010). They also increase the uncertainty of predictions. This analysis has shown the suitability of GAMLSS for extracting information and improving predictions related to processes obscured by synergistic interactions, and for differentiating local from broad regional climatic impacts.

This study also further demonstrates the capacity of herbarium collections to shed light on changing synchronies between interacting species at a given location under ongoing climate change (Prather, Alvarez-Fuentes et al. 2004; Suarez and Tsutsui 2004). While the current study indicates the importance of seasonal rain, the impact of breaking rains in relation to extended drought periods should be an important focus in future investigations (Opler, Frankie et al. 1976; Bowers 2007). Climatic drivers of orchid phenology may be further elucidated by GAMLSS modelling of peak flowering date with respect to lagged monthly climate prior to flowering (Hudson, Kim et al. 2011a) in addition to seasonal climatic predictors via GAMLSS or a mixed transition state approach (Hudson, Kim et al. 2011b) or wavelet (Hudson, Keatley et al. 2011a; Hudson, Keatley et al. 2011b; 2011c; Hudson, Kim et al. 2011a) approaches.



***Acknowledgements***

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## **Supplementary material for Chapter 3 -**

submitted as

**Supporting Online material for *Climatic Change* paper**

### **Supplementary material**

- S1     *Diuris species and location*
- S2     *GAMLSS models, analysis and interpretation*
- S3     *Global climatic drivers of flowering phenology*

## S1 Study site and species

The Australian terrestrial orchid genus *Diuris* comprises at least 61 species (Indsto 2009; Indsto, Weston et al. 2009), and derives its name with reference to the distinctive lateral sepals (Greek: *dis*, two; *oura*, a tail). Following a period of summer aestivation, shoots emerge from underground tubers, and in spring conspicuous yellow flowers present a pair of pollinia for transfer. These orchids employ a deceptive pollination strategy (Ramírez, Eltz et al. 2011) and time their mass flowering to coincide with the beginning of the flowering season of the Australian pea family, Fabaceae (Indsto 2009, personal observations). Utilizing floral mimicry, they attract the introduced honey bee, native bees and wasps (Indsto 2009; Indsto, Weston et al. 2009) in search of the abundant nectar and pollen provided by the target pea flowers (Beardsell, Clements et al. 1986). The three orchid species of this study have a wide distribution throughout much of southern South Australia (Figures S1.1 & S1.2), growing in heathland, woodland or open eucalypt forest, and can often be found growing in close proximity and flowering synchronously (Figures S1.2 & S1.3).

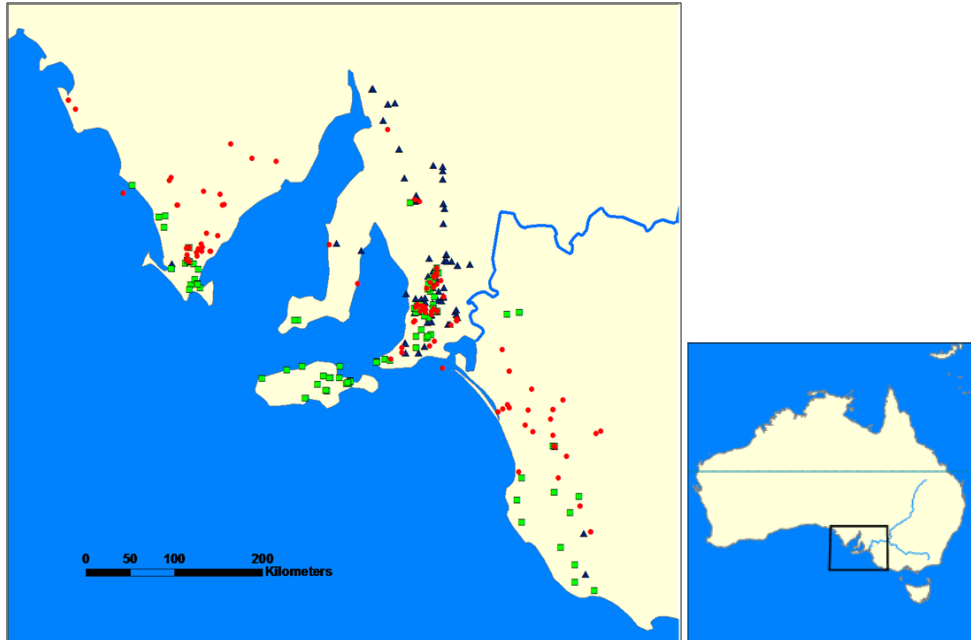
Winter and spring climatic events contribute differently to the orchids' reproductive cycle: winter conditions impact on the pre-flowering stages of the plants' development, such as vegetative growth and flower initiation (Blanchard and Runkle 2006), whereas spring rain has a greater contribution to capsule and seed development, recruitment, and tuber resources, with effects manifesting the following year (Pfiefer, Heinrich et al. 2006). Advanced flowering under drought conditions may be a response which allows these *Diuris* species to complete their reproductive cycle before soils dry out. Extended drought may have further implications relating to the size and vigour of the plant (field observations) in addition to shifts in flowering phenology. While the tuberous root system may safeguard these orchids during years of low rainfall, the altered phenological response of all species to later flowering under extremely dry conditions suggests that extended periods of severe drought may have a disproportionate influence. Additional constraints on the overall extent of a flowering season arise from the increased risk of frost damage to early flowering plants, with effects exacerbated by increasing aridity (Pfiefer,

Heinrich et al. 2006), and heat waves in spring which result in the abrupt end of a flowering event (my personal observation, Belair National Park, 2009).

Large and consistent temporal differences among locally adjacent, but distinct, populations (personal observation) indicate a local pool of genetic resources upon which natural selection can act. The distribution of these resources through the process of hybridization (Cozzolino, Nardella et al. 2006; Buggs 2007) will ensure a supply of individuals with an improved capacity to cope with the more extreme conditions imposed by future change (Seehausen 2004; Blanchard and Runkle 2006; Hoffmann and Sgrò 2011). If reproductive processes are negatively affected through disruptions of synchrony between sympatric species and their pollinators, this potentially valuable resource may be lost. Ensuing population decline may be postponed in the short term due to the longevity of individuals (Miller-Rushing, Høye et al. 2010), their clonal capabilities, and their ability to self-fertilize to produce viable offspring. For these food deceptive orchids, corresponding phenological shifts of Fabaceae species will also be influential on long-term reproductive success.



**Fig. S1.1** Google Earth view of the South Australian study area with latitudes 34.5°S, 35.5°S and longitudes 138°E, 139°E highlighted in yellow. These longitudes bound the Mt Lofty Ranges to the east and west, and Adelaide is situated in the central region. The four yellow pins mark the geographical limits of the three study species. Their actual locations are shown in Figure S1.2.



**Fig. S1.2** Mapped locations represented by the herbarium specimens on which this *Diuris* analysis is based. There is an absence of data in the north eastern region (Longitude 3: Latitude 3); Kangaroo Island is located in the south-western region (Longitude 1: Latitude 1). The majority of specimens are representative of the central Mt. Lofty region (Longitude 2:Latitude 2) where all three species can often be found growing in close proximity and flowering synchronously.

*D. pardina* ●    *D. orientis* ■    *D. behrii* ▲





**Fig. S1.3** *Diuris* orchids flowering at Belair National Park. Clockwise from top: *D. pardina*; *D. behrii* x *orientis* hybrid; *D. orientis*; *D. behrii*



## **S2. Generalized Additive Models for Location, Scale and Shape (GAMLSS), analysis and interpretation**

S2.1 Generalized Additive Models for Location, Scale and Shape (GAMLSS) were introduced by Rigby and Stasinopoulos (2005) to overcome various limitations of Generalized Linear Models (Nelder and Wedderburn 1972) and Generalized Additive Models (Hastie and Tibshirani 1999; Hastie 2008; Gair, Dhar et al. 2011). The method provides a general framework for univariate regression analysis which allows for the modelling of parameters (e.g. mean or location) using non-parametric smoothing functions such as cubic splines. GAMLSS can deal with continuous and discrete distributions which are highly skewed or kurtotic and allow each regression variable to have a non-linear relationship with the dependent variable (Stasinopoulos and Rigby 2007). The type of non-linearity is not specified a priori, but is calculated and tested for in the modelling process. The GAMLSS framework is implemented in a series of packages in R (R Development Core Team 2007), a free software (see URL <http://www.R-project.org>).

GAMLSS have been used to estimate both long and short-term non-linear phenological effects of climate, identify change-points and thresholds for earliest and latest flowering times, and account for the auto-correlated nature of a phenological series by incorporating lag effects (Hudson, Kim et al. 2010; 2011a). They were recently proposed as one approach for meta-analytic studies of change in phenology with respect to climate (Hudson 2010b) and to model climatic impacts on the flowering phenology of eight eucalypt species (Hudson, Kim et al. 2011a).

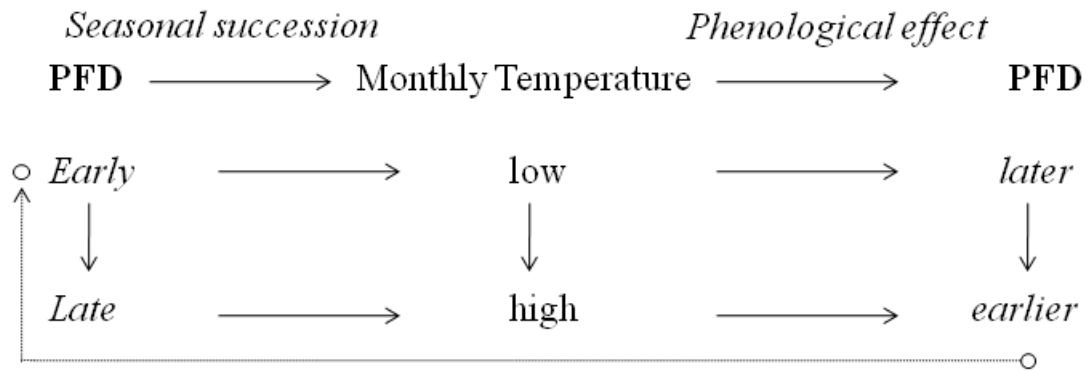
For this study GAMLSS were used as a curve fitting modelling tool to identify the main climatic drivers of change in flowering phenology from a range of climatic predictors. Each model assumes that the flowering series represents normally distributed data and the RS algorithm, a generalization of the algorithm of Rigby and Stasinopoulos (1996) was used to obtain the estimates of the predictor effects. Twelve initial exploratory models of increasing complexity were built by adding together combinations of Year, Species, Season, Altitude, Longitude, Latitude and four climatic parameters, mean minimum

temperature (monthly and seasonal) and rainfall (monthly and seasonal), as predictors of flowering time (Table S2.1). Monthly and seasonal temperatures and rainfall correspond to the month and season in which flowering occurred. Each term in a given model shows the effect of a given predictor after adjusting for the effects of all other predictors in the model. Term plots of the main effects obtained in the principal reference model (PRM) are shown in Fig. S2.2. The effects of year, temperature, rainfall, and altitude are to be understood as cubic spline (cs) effects with associated non-linear effects and given P-values. Goodness of fit was assessed by the Akaike Information Criterion (AIC) (Akaike 1983) with lowest AIC values indicating optimal models, and the diagnostic plots of residuals and fitted values were used to check for systematic bias or lack of fit in the main effects component of the models (Fig. S2.3).

All models were run in two versions, addressing main effects both separately and in combination with two-way interaction effects (Tables S2.2, S2.3). The baseline of contrasts for the categorical predictors are as follows: *D. behrii* for species; winter for season; the western region (level 1) for longitude and the southern region (level 1) for latitude. Interaction plots, which show differential changes in the strength and/or direction of the relationship between term contrasts and the outcome peak flowering day (PFD), are only available when at least one of the terms is categorical, and not for interactions between two continuous covariates e.g. temperature and rainfall.

Optimal models as identified by the AIC as the most powerful descriptors of the flowering time series patterns incorporated both monthly and seasonal climate variables along with season, year, species, altitude, with or without longitude and latitude (Table S2.2). However, with month of flowering unaccounted for, the monthly estimates obtained from these models are misleading as they do not simply describe a phenological response to climate; later flowering events in October and November occur during a period of higher monthly temperatures and lower monthly rainfall than do early flowering events, generating a statistically circular argument as noted by (Primack, Ibáñez et al. 2009) (Fig. S2.1). As such, PFD functions as a time of year equivalent leading to large and positive values for monthly temperature ( $\beta=+6.8$ ;  $P=0.000$ ) and negative values for monthly

rainfall ( $\beta=-0.14$ ;  $P=0.000$ ) (Table 1). Correlations of monthly climate indicators with month of peak flowering (mean minimum monthly temperature:  $\rho=0.52$ ,  $P=0.000$ ; monthly rainfall:  $\rho=-0.36$ ,  $P=0.000$ ; ( $\rho$  Spearman's rank correlation coefficient) confirm this strong directional signal which masks the underlying phenological response of shifts to *earlier* flowering in *seasons* with higher temperatures and/or lower rainfall). Monthly climatic variables have thus been considered here as proxies for the month of flowering and not potential drivers of flowering phenology. While these monthly inclusions increase both the size and statistical significance of seasonal climatic estimates as main effects, the predictions of this model are interpreted with caution for reasons outlined above.



**Fig. S2.1** Diagrammatic representation of the interrelated dual aspects of seasonal succession and phenological response implicit in phenological time-series data sets. Temperature and rainfall not only influence phenology but also vary with the timing of flowering events; e.g. early flowering occurs in the colder months of July and August; late flowering occurs in the warmer months of October and November. This is not a phenological response to climate; monthly climate is simply acting as a time proxy for peak flowering day. Simultaneously, but independently, a phenological response results in shifts to earlier flowering in seasons with higher temperatures/lower rainfall (as the explanatory variables). A negative feedback effect is evident:  $\bigcirc \longleftarrow \bigcirc$

**Table S2.1** 12 main effects models: all models estimate peak flowering day (PFD) and incorporate Year, Species and Altitude. All temperatures are mean minimum temperatures; rainfall is cumulative total. x: the predictor included in model. Model 10 is the PRM, model 11 the SM1 and model 4 the SM2

Model	Season	Monthly Temperature	Monthly Rainfall	Seasonal Temperature	Seasonal Rainfall	Latitude & Longitude
1		x	x			
2				x	x	
3	x	x	x			
4	x			x	x	
5	x	x	x	x	x	
6		x	x	x	x	
7		x	x			x
8				x	x	x
9	x	x	x			x
10	x			x	x	x
11	x	x	x	x	x	x
12		x	x	x	x	x

**Table S2.2** Significance of the 12 main effects models; << : P-value much less than; NS: not significant at  $P=0.1$ ; \* cubic spline; GD: Global Deviance; AIC: Akaike Information Criterion ; SBC: Schwartz Bayesian Criterion.

Main effects	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
Intercept	<<0.001	<0.001	<<0.001	0.006	<0.001	<0.001
Year*	<<0.001	0.002	<<0.001	NS	NS	<0.001
<i>D. orientis</i> vs. <i>D. behrii</i>	NS	<0.001	0.100	<0.001	0.019	0.013
<i>D. pardina</i> vs. <i>D. behrii</i>	NS	NS	NS	NS	NS	NS
Season (winter vs. spring)			<<0.001	<<0.001	<<0.001	
Altitude*	NS	NS	NS	NS	NS	NS
Temperature* (monthly)	<<0.001		<<0.001		<<0.001	<<0.001
Rain* (monthly)	<0.001		<0.001		<0.001	<0.001
Temperature* (seasonal)		0.050		0.003		<0.001
Rainfall* (seasonal)		NS		NS		0.003
GD:	2212.8	1888.8	2172.8	2246.2	2172.8	2071.5
AIC:	2252.8	2076.8	2214.8	2288.2	2214.8	2127.5
SBC:	2326.3	2421.8	2292.0	2365.3	2292.0	2230.3

Main effects	Model 7	Model 8	Model 9	Model 10	Model 11	Model 12
Intercept	<<0.001	<0.001	<<0.001	0.008	<0.001	<0.001
Year*	<<0.001	0.002	<<0.001	NS	NS	<0.001
<i>D. orientis</i> vs. <i>D. behrii</i>	0.047	<0.001	0.008	<0.001	0.006	0.004
<i>D. pardina</i> vs. <i>D. behrii</i>	NS	NS	NS	NS	NS	NS
Season (winter vs. spring)			<<0.001	<<0.001	<<0.001	
Altitude*	NS	0.070	NS	0.061	NS	NS
Temperature* (monthly)	<<0.001		<<0.001		<<0.001	<<0.001
Rain* (monthly)	<0.001		<0.001		<0.001	<0.001
Temperature* (seasonal)		NS		0.002	<<0.001	<0.001
Rainfall* (seasonal)		NS		0.068	<0.001	0.004
Longitude (central vs. west)	NS	NS	NS	NS	NS	NS
Longitude (east vs. west)	<0.001	NS	<0.001	NS	0.011	0.005
Latitude (central vs. south)	0.013	NS	0.012	NS	NS	NS
Latitude (north vs. south)	0.019	NS	0.028	NS	NS	NS
GD:	2190.4	2259.0	2150.5	2241.4	2027.4	2056.8
AIC:	2238.4	2307.0	2200.5	2291.4	2093.4	2120.8
SBC:	2326.5	2395.1	2292.2	2383.2	2214.5	2238.2

**Table S2.3a** Estimates and significance of main effects when interactions are taken into account. Estimates are given for the principal reference model (PRM), support model 1 (SM1) and support model 2 (SM2); \* cubic splines; NS: not significant.

	PRM		SM1		SM2	
	Estimate	Pr(> t )	Estimate	Pr(> t )	Estimate	Pr(> t )
Intercept	-1100	NS	-521	NS	-4870	<<0.001
Year*	0.764	NS	0.236	NS	2.65	<<0.001
Species <i>orientis</i> vs. <i>D. behrii</i>	128	NS	-186	NS	143	NS
Species <i>pardina</i> vs. <i>D. behrii</i>	-179	NS	-81.4	NS	-55.3	NS
Altitude*	-0.543	NS	-0.567	NS	-0.546	NS
Temperature*(monthly)			21.1	NS		
Rain*(monthly)			-3.02	NS		
Longitude (central vs. west)	-228	NS	78.1	NS		
Longitude (east vs. west)	-865	0.095	-11.3	NS		
Latitude (central vs. south)	-345	NS	471	0.010		
Latitude (north vs. south)	-269	NS	802	0.021		
Season (winter vs. spring)	1210	NS	2710	NS	861	0.081
Temperature*(seasonal)	194	0.011	26.0	NS	452	<<0.001
Rain*(seasonal)	-2.33	NS	1.25	NS	0.266	NS

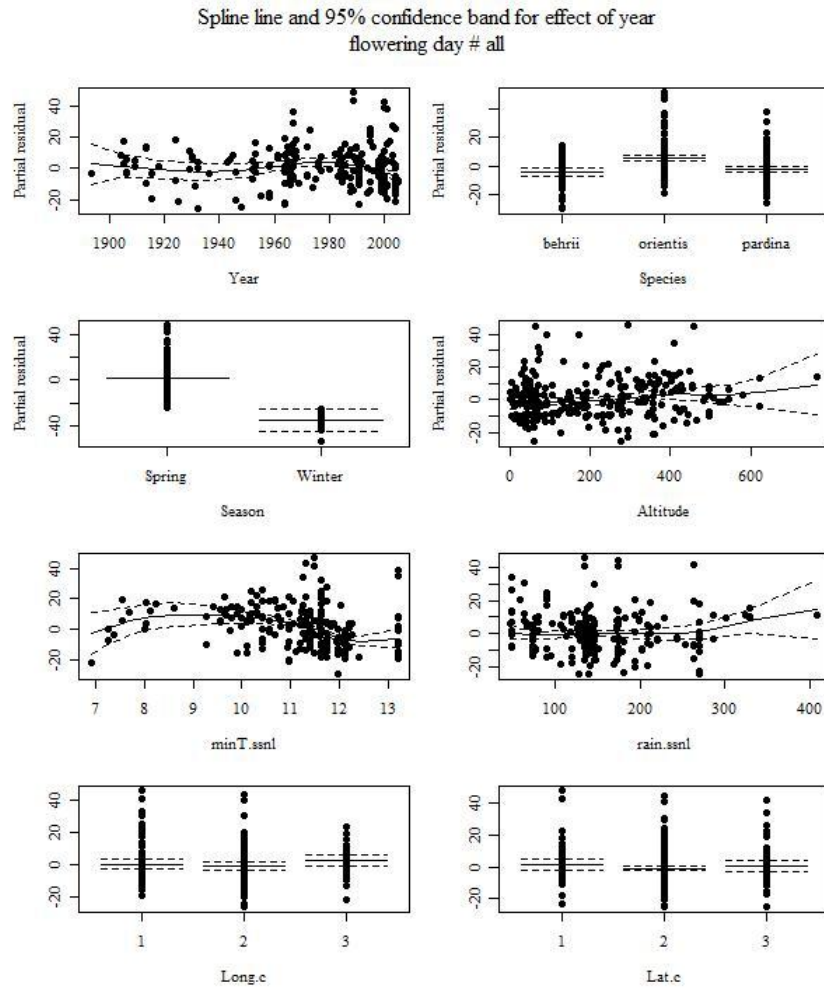
**Table S2.3b** Estimates and significance of interactive effects for the principal reference model (PRM), support model 1 (SM1) and support model 2 (SM2); \* cubic splines;  
NS: not significant at 10%; GD: Global Deviance; AIC: Akaike Information Criterion; SBC: Schwartz Bayesian Criterion.

	PRM		SM1		SM2	
	Estimate	Pr(> t )	Estimate	Pr(> t )	Estimate	Pr(> t )
Year*: <i>D. orientis</i> vs. <i>D. behrii</i>	-0.074	NS	0.134	NS	-0.095	NS
Year*: <i>D. pardina</i> vs. <i>D. behrii</i>	0.070	NS	0.059	NS	0.006	NS
Year*: Season (winter vs. spring)	-0.826	NS	-1.74	NS	-0.560	0.055
Year*: Altitude*	0.000	NS	0.000	NS	0.000	NS
Year*: Temperature*(monthly)			0.002	NS		
Year*: Rain*(monthly)			0.002	NS		
Year*: Temperature*(seasonal)	-0.112	0.005	-0.008	NS	-0.241	<<0.001
Year*: Rain*(seasonal)	0.001	NS	-0.001	NS	-0.000	NS
Year*: Longitude (central vs. west)	0.011	NS	-0.040	NS		
Year*: Longitude (east vs. west)	0.422	0.019	0.052	NS		
Year*: Latitude (central vs. south)	0.115	NS	-0.235	NS		
Year*: Latitude (north vs. south)	0.081	NS	-0.380	0.038		
<i>D. orientis</i> vs. <i>D. behrii</i> : Season (winter vs. spring)	14.8	NS	107	NS	-12.4	NS
<i>D. pardina</i> vs. <i>D. behrii</i> : Season (winter vs. spring)	41.0	NS	120	NS	2.16	NS
<i>D. orientis</i> vs. <i>D. behrii</i> : Altitude*	0.052	0.039	0.040	0.017	0.022	0.097
<i>D. pardina</i> vs. <i>D. behrii</i> : Altitude*	-0.007	NS	0.014	NS	-0.018	NS
<i>D. orientis</i> vs. <i>D. behrii</i> : Temperature*(monthly)			0.727	NS		
<i>D. pardina</i> vs. <i>D. behrii</i> : Temperature*(monthly)			2.67	0.062		
<i>D. orientis</i> vs. <i>D. behrii</i> : Rain*(monthly)			0.005	NS		
<i>D. pardina</i> vs. <i>D. behrii</i> : Rain*(monthly)			0.072	NS		
<i>D. orientis</i> vs. <i>D. behrii</i> : Temperature*(seasonal)	1.68	NS	-5.69	0.028	3.63	NS
<i>D. pardina</i> vs. <i>D. behrii</i> : Temperature*(seasonal)	4.05	NS	-4.48	0.074	3.46	NS
<i>D. orientis</i> vs. <i>D. behrii</i> : Rain*(seasonal)	0.046	NS	-0.015	NS	0.075	0.055

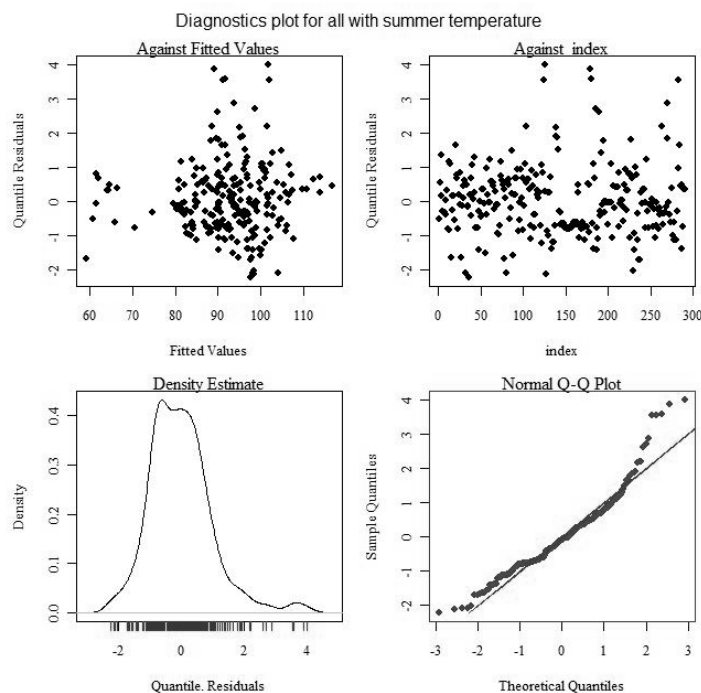


<i>D. pardina</i> vs. <i>D. behrii</i> : Rain*(seasonal)	0.074	0.099	0.012	NS	0.071	0.077
<i>D. orientis</i> vs. <i>D. behrii</i> : Longitude (central vs. west)	-3.80	NS	-22.3	0.020		
<i>D. pardina</i> vs. <i>D. behrii</i> : Longitude (central vs. west)	14.8	NS	-23.2	0.022		
<i>D. orientis</i> vs. <i>D. behrii</i> : Longitude (east vs. west)	23.5	NS	-13.6	NS		
<i>D. pardina</i> vs. <i>D. behrii</i> : Longitude (east vs. west)	14.6	NS	-8.54	NS		
<i>D. orientis</i> vs. <i>D. behrii</i> : Latitude (central vs. south)	-10.7	NS	-6.88	NS		
<i>D. pardina</i> vs. <i>D. behrii</i> : Latitude (central vs. south)	-31.1	0.032	-1.56	NS		
<i>D. orientis</i> vs. <i>D. behrii</i> : Latitude (north vs. south)	-29.6	NS	-10.6	NS		
<i>D. pardina</i> vs. <i>D. behrii</i> : Latitude (north vs. south)	-35.9	0.031	-5.22	NS		
Season (winter vs. spring): Altitude*	-0.076	NS	0.002	NS	0.045	NS
Temperature*(monthly): Season (winter vs. spring)			22.0	NS		
Rain*(monthly): Season (winter vs. spring)			-0.392	NS		
Season (winter vs. spring): Temperature*(seasonal)	23.9	NS	32.6	0.010	19.0	NS
Season (winter vs. spring): Rain*(seasonal)	0.045	NS	0.595	NS	0.121	NS
Season (winter vs. spring): Longitude (central vs. west)	32.5	NS	-1.91	NS		
Season (winter vs. spring): Longitude (east vs. west)	-4.78	NS	27.8	NS		
Season (winter vs. spring): Latitude (central vs. south)	139	0.069	21.9	NS		
Season (winter vs. spring): Latitude (north vs. south)	135	0.068				
Altitude*: Temperature*(monthly)			-0.001	NS		
Altitude*: Rain*(monthly)			0.000	NS		
Altitude*:Temperature*(seasonal)	-0.020	NS	-0.005	NS	0.021	0.007
Altitude*:Rain*(seasonal)	0.000	NS	-0.000	NS	0.000	0.002
Altitude*: Longitude (central vs. west)	-0.028	NS	-0.006	NS		
Altitude*: Longitude (east vs. west)	-0.038	NS	0.006	NS		
Altitude*: Latitude (central vs. south)	-0.005	NS	0.035	0.068		
Altitude*: Latitude (north vs. south)	-0.000	NS	0.052	0.060		
Temperature*(monthly): rain*(monthly)			-0.077	0.002		

Temperature*(monthly):Temperature*(seasonal)			-1.94	0.005		
Temperature*(monthly): Rain*(seasonal)			0.057	<<0.001		
Temperature*(monthly): Longitude (central vs. west)			-6.52	<0.001		
Temperature*(monthly): Longitude (east vs. west)			-5.87	0.004		
Temperature*(monthly): Latitude (central vs. south)			3.87	0.071		
Temperature*(monthly): Latitude (north vs. south)			4.16	0.068		
Rain*(monthly): Temperature*(seasonal)			-0.047	NS		
Rain*(monthly): Rainfall*(seasonal)			0.001	0.013		
Rain*(monthly): Longitude (central vs. west)			0.120	NS		
Rain*(monthly): Longitude (east vs. west)			0.029	NS		
Rain*(monthly): Latitude (central vs. south)			0.008	NS		
Rain*(monthly): Latitude (north vs. south)			-0.027	NS		
Temperature*(seasonal): Rain*(seasonal)	0.018	NS	0.018	NS	0.051	0.019
Temperature*(seasonal): Longitude (central vs. west)	15.9	<0.001	8.74	0.010		
Temperature*(seasonal): Longitude (east vs. west)	0.353	NS	-0.808	NS		
Temperature*(seasonal): Latitude (central vs. south)	11.0	0.025	-3.46	NS		
Temperature*(seasonal): Latitude (north vs. south)	11.9	0.054	-6.19	NS		
Rain*(seasonal): Longitude (central vs. west)	0.071	NS	-0.016	NS		
Rain*(seasonal): Longitude (east vs. west)	-0.065	NS	-0.048	NS		
Rain*(seasonal): Latitude (central vs. south)	-0.080	NS	-0.023	NS		
Rain*(seasonal): Latitude (north vs. south)	-0.104	NS	-0.047	NS		
Longitude (central vs. west): Latitude (central vs. south)	11.8	NS	-17.5	0.016		
Longitude (east vs. west): Latitude (central vs. south)	26.3	0.021	-8.19	NS		
Longitude (central vs. west): Latitude (north vs. south)	-11.4	NS	-40.8	<0.001		
GD:	2137.5		1828.7		2195.0	
AIC:	2289.5		2040.7		2277.0	
SBC:	2568.4		2429.7		2427.4	



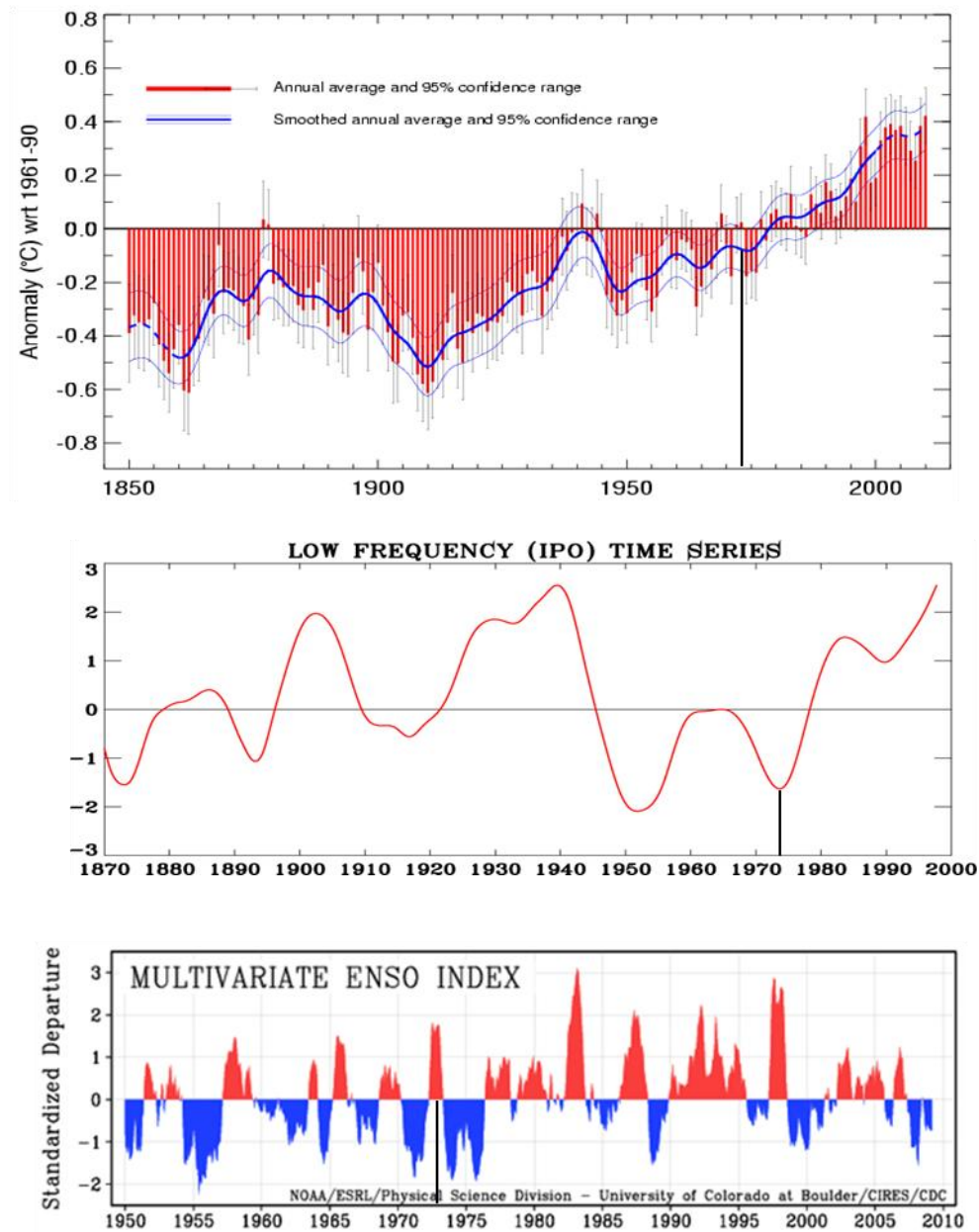
**Fig. S2.2** Term plots of the main effects in model 10, the principal reference model (PRM). Estimates and significance of all terms are given in Table 1. The GAMLSS spline line and 95% confidence band show the effects of mean minimum seasonal temperature (minT.ssnl) and seasonal rainfall (rain.ssnl) on peak flowering date (PFD) for combined species. On average, PFD advanced by  $\sim 3.5$  days  $^{\circ}\text{C}^{-1}$  rise in seasonal minima ( $P=0.002$ ). There is also some evidence of an advance of  $\sim 2$  days  $100\text{mm}^{-1}$  decrease in seasonal rainfall ( $P=0.068$ ). Species, season, and seasonal climate are significant main effects. Year, Longitude and Latitude are not significant as main effects but Altitude is significant at 10% ( $P=0.06$ ) in this model.



**Fig. S2.3** Diagnostic plots for the main effects in the principal reference model (PRM). The GAMLSS fit of the data shown in the Normal Q-Q Plot of the theoretical quantiles is less optimal than for models which were biased by the inclusion of monthly analogues (Fig.S2.1) and thus were considered inappropriate for consideration.

### S3 Global climatic drivers of flowering phenology

As discussed in the Introduction and Conclusions of the main text, global weather systems are the ultimate drivers of flowering phenology of these *Diuris* orchid populations. Fig. S3.1 shows how the overall trend to earlier flowering approximates the pattern of increase in global average sea-surface temperatures over the corresponding period. GAMLSS change point years 1932/33 and 1973/74 distinguish two distinct episodes of rapid flowering advancement, separated by a period in the middle of the century when flowering time advanced little, and was even delayed in the eastern part of the range. This was a period during which sea-surface temperatures fell, the IPO showed a significant decline and La Niña phases predominated. The most recent rapid rate of advancement subsequent to the change point in 1973 (Fig. 2) approximates the beginning of the shift to a major positive phase of the IPO and the corresponding prolonged record breaking El Niño phase.



**Fig. S3.1** Top: Global average sea-surface temperature based on Rayner, Brohan et al. (2006) <http://www.metoffice.gov.uk/hadobs/hadcrut3/diagnostics/comparisoN.H.html> (contains public sector information licensed under the Open Government Licence v1.0); centre: the Inter-decadal Pacific oscillation <http://www.bom.gov.au/climate/cli2000/jimSal.html>; bottom: the Multivariate ENSO index. The vertical line marking the 1973 GAMLSS change point approximates the beginning of above average sea-surface temperatures, the shift to a major positive phase of the IPO and the corresponding record breaking El Niño phase. Change point for 1932/33 marking the end of a considerable advancement in PFD early in the 20th century has no counterpart in these plots.



## Chapter 4 *Pyrus* phenology in South Australia

Phyllis F. MacGillivray

*School of Earth and Environmental Sciences, Australian Centre for Evolutionary Biology and Biodiversity, The University of Adelaide, South Australia, Australia*

Phone +61 8 8303 5148

Fax +61 8 8303 4364

Email [phyllis.macgillivray@adelaide.edu.au](mailto:phyllis.macgillivray@adelaide.edu.au)

Andrew J. Lowe

*School of Earth and Environmental Sciences, Australian Centre for Evolutionary Biology and Biodiversity, The University of Adelaide, South Australia, Australia and*

*State Herbarium of South Australia, Science Resource Centre, Department of Environment and Natural Resources, South Australia, Australia.*

This chapter is dedicated to the late Dr. David Symon (1920 – 2011), who was personally responsible for the establishment of the pear plantation at the Waite Arboretum and the phenological recordings on which this work is based.





## Statement of Authorship

Title of Paper	<i>Pyrus</i> phenology in South Australia
Publication Status	<input type="radio"/> Published <input type="radio"/> Accepted for Publication <input type="radio"/> Submitted for Publication <input checked="" type="radio"/> Publication Style
Publication Details	This paper has been prepared as a submission for publication

### Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Phyllis (Fran) MacGillivray		
Contribution to the Paper	Sought and obtained funding; prepared data set for analysis; performed analysis; wrote manuscript as principal author		
Signature		Date	26 Oct 2012

Name of Co-Author	Andrew J Lowe		
Contribution to the Paper	As principal supervisor, sought and accessed data; advised on development of the manuscript.		
Signature		Date	23.10.12



*“Outside the courtyard but stretching close up to the gates, and with a hedge running down on either side, lies a large orchard of four acres, where trees hang their greenery on high, the pear and the pomegranate, the apple with its glossy burden, the sweet Fig and the luxuriant olive. It comes at all seasons of the year, and there is never a time when the West Wind’s breath is not assisting, here the bud, and here the ripening fruit; so that pear after pear, apple after apple, cluster on cluster of grapes, and Fig upon Fig are always coming to perfection.*

*..... Such were the beauties with which the gods had adorned Alcinous’ home.”*

Homer ‘The Odyssey V11 p 115 (Radice 1946)<sup>5</sup>

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<sup>5</sup> Attributed to Homer - somewhere in the Ionian region of Asia Minor in the 7th or 8th centuries BC.

## Abstract

A 44-year replicate dataset (1967-2010) of 112 *Pyrus* (pear) trees growing at the Waite Arboretum in Adelaide provided a unique resource for investigating the flowering phenology of non-native, deciduous species subject to identical climatic conditions. Multiple linear regression was used to investigate minimum temperature and rainfall as drivers of phenology at the genus and species levels; boxplots and one-way ANOVA identified similarities and differences among and between species and individual trees. Peak flowering occurred in each month from May to October. The longest range for a single tree was 122 days; the shortest, 19 days. A highly significant response of the genus to the lowest mean minimum monthly temperature for the year ( $T_m$ ) ( $\beta = -7.13 \pm 1.21$ ;  $P < 0.000$ ) represents an advance of around 7 days for each  $^{\circ}\text{C}$  increase in  $T_m$ . The month in which this minimum temperature occurred was also highly significant ( $\beta = 11.69 \pm 1.84$ ;  $P < 0.000$ ). In addition, for each month earlier in the year  $T_m$  occurred, peak flowering advanced approximately  $11\frac{1}{2}$  days. Exposure to summer temperatures above  $38^{\circ}\text{C}$  initiated an unseasonal flowering event. Specific rainfall patterns had a further substantial influence, with autumn flowering episodes frequently triggered by breaking rains following a period of extended drought. *Pyrus* respond to the climatic extremes encountered in southern South Australia with extreme variation in flowering phenology and an associated, potentially high, economic cost to the horticultural industry. The various species and cultivars have their own distinctive constraints and triggers, uniquely influenced by genotype.

**Keywords:** *Pyrus*; climate; drought; temperature; rainfall; extreme climatic events; South Australia

## Introduction

It is imperative that the timing of important phenological events synchronize with seasonal conditions conducive to successful reproduction. To this end, plants have evolved reliable environmental monitoring and regulatory response mechanisms to survive the climatic vagaries of their native environment (Balasubramanian, Sureshkumar et al. 2006; Campoy, Ruiz et al. 2011). Intra- and interspecific variability caters to a wide range of climatic conditions, and ensures that flowering occurs under the most favourable circumstances for reproduction and seed dispersal (Jones, Hecht et al. 2011).

The flowering response to temperature is regulated by the dual processes of vernalization and dormancy which, together, optimize opportunities for growth while affording protection from the deleterious effects of protracted low temperatures (Heide and Prestrud 2005). Vernalization is linked to the physiology of *reproduction*, and ensures that progression to flowering is suspended during a sufficient period of cold to mark the passage of winter and the arrival of spring (Amasino 2004). Dormancy is concerned with vegetative development (Chouard 1960) and comprises a range of physiological processes which respond to, and are regulated by, photoperiodic and seasonal environmental cues (Horvath, Anderson et al. 2003; Campoy, Ruiz et al. 2011). Vernalization thus induces the *capacity* for flowering, but floral initiation only occurs after exposure to warmer conditions and the release of dormancy (Chouard 1960; Simpson and Dean 2002).

In cold climates, bud initiation actually begins at the end of the growing season as summer days shorten, and peaks one to two months after the autumn equinox (Horvath, Anderson et al. 2003; Tanino, Kalcsits et al. 2010; Campoy, Ruiz et al. 2011). The advent of colder weather signals the winter dormancy phase, which prevents further development until the return of favourable conditions (Chouard 1960; Opler, Frankie et al. 1976; Battey 2000). Chilling requirements are highly variable and dependent upon location and cultivar (Campoy, Ruiz et al. 2011). Dormancy and flowering are regulated by similar signals, with an overlap in the physical triggers regulating both processes (Horvath, Anderson et al. 2003).

For plants growing at lower latitudes, relatively warm winters provide year round growing conditions, and the phenological constraint imposed by the shorter reproductive season at higher latitudes (Bolmgren and Lönnberg 2005) does not apply. On the other hand, plants must withstand periods of extreme summer heat, often exacerbated by severe and prolonged restricted water availability. Campoy et al. (2011) speculate that fruit trees adapted to these latitudes, where temperatures vary more strongly than daylength (Battey 2000), have greater reliance on temperature sensitive mechanisms than on photoperiodic responses to time their phenology (Luedeling, Gebauer et al. 2009; Tanino, Kalcsits et al. 2010; Campoy, Ruiz et al. 2011). Corroborating evidence of flowering shifts by four days/degree of latitude of the pear, *Pyrus communis*, is provided by Sparks and Menzel (2002), reinforcing the influence of the latitudinal temperature gradient on flowering time.

Alternative dormancy-release strategies are needed where chill requirements are not satisfied (Yu, Luedeling et al. 2010). Genetic differences, both intra- and interspecific, provide for a range of responses (Campoy, Ruiz et al. 2011; Jones, Hecht et al. 2011) evident across latitudinal ecotypes (Myking and Heide 1995). Heide and Prestrud (2005) report an inverse correlation of inherent dormancy period with latitude of origin: the short photoperiods requisite for dormancy in northern ecotypes of dogwood hybrids are not required by southern ecotypes (Tanino, Kalcsits et al. 2010). For many rosaceous tree species, such as the apple and pear, temperatures lower than 12<sup>0</sup>C are sufficient for dormancy induction, independent of daylength (Heide and Prestrud 2005; Olsen 2010; Tanino, Kalcsits et al. 2010; Campoy, Ruiz et al. 2011).

While it has become an accepted and well established view that increasing temperatures prior to flowering lead to notable advances in spring phenophases for the *majority* of species (Fitter and Fitter 2002; Wolfe, Schwartz et al. 2005; Doi 2007; Guédon and Legave 2008; Yu, Luedeling et al. 2010), there is a growing body of evidence that reduced chilling during warmer winters *delays* the onset of flowering due to the longer pre-flowering period needed to compensate for vernalization processes which are inhibited above requisite low temperatures (Cave and Johnston 2010). In combination, these

temperature-related effects reduce the length of the flowering season (Yu, Luedeling et al. 2010; Campoy, Ruiz et al. 2011; Okie and Blackburn 2011).

In the tropical conditions of Costa Rica, where daily temperature extremes exceed those of monthly differences, Opler, Frankie et al. (1976) found it was rainfall in the latter half of the dry season which had an overriding influence on flowering. With progression to flowering dependent on highly unpredictable rainfall events, it follows that the timing of this phenophase will be likewise unpredictable, and there is a good possibility that plants may get it wrong. In Costa Rica, some species failed to flower at all in some years and responded with multiple flowerings in other years. Multiple flowering events resulted when the first flowering episode was terminated by a further dry period. A later rain event triggered a second flowering, especially in trees with poor initial fruit set (Opler, Frankie et al. 1976).

### ***Pyrus phenology***

*Pyrus* are woody deciduous trees of the Rosaceae family with origins in southern Europe and Asia. Seasonal phenophases reportedly progress independently of photoperiod (Heide and Prestrud 2005; Olsen 2010; Tanino, Kalcsits et al. 2010; Campoy, Ruiz et al. 2011), and no other alternative environmental signal is known to control their dormancy. They are, however, highly responsive to prevailing minimum temperatures (Heide and Prestrud 2005). Following 3000 years of domestication (Radice 1946), *Pyrus* species are now represented globally by numerous horticultural varieties. In many of the traditional fruit growing regions, this economically important food crop is now under threat from climate change.

The genus features a clear separation of floral initiation and subsequent flowering stages (Banno, Hayashi et al. 1986). In Osaka, Japan, floral initiation for the following year in *Pyrus pyrifolia* begins in late June, approximately 75 days after the period of full bloom. Differentiation is completed by early July after which further development is suspended until the following spring (Esumi, Tao et al. 2007). Pears have one of the highest chilling

requirements of all fruit trees (600 hours for *P. communis* L.), second highest only to plum *Prunus domestica* (Luedeling, Gebauer et al. 2009). Growth cessation and dormancy induction in *P. communis* can be induced by temperatures below 12°C, regardless of photoperiodic conditions (Heide and Prestrud 2005), but satisfactory dormancy release and flowering have more stringent requirements. When these conditions are not met, trees are deficient in flowering and fruit set (De Melo-Abreu, Silva et al. 2005).

The various fruit tree species and cultivars have their own specific heat requirements for dormancy release and flowering (De Melo-Abreu, Silva et al. 2005), but selection of suitable genotypes for production in foreign climates often involves compromise. Cultivars with lower chilling requirements, selected for their superior ability to cope with warmer environments, remain susceptible to erratic fruit set, and warming in autumn has been found to slow the rate of subsequent flower development in *P. communis* cv. Concorde, although the delay was overcome by full flowering stage (Esumi, Tao et al. 2007). Elevated autumn temperatures can also lead to autumn flowering (Campoy, Ruiz et al. 2011), and exposure to extreme heat can short-circuit vernalization mechanisms in *P. communis* and *P. pyrifolia* with subsequent episodes of flowering (Tamura, Tanabe et al. 1998).

Hence, exceptional weather conditions may be variously detrimental to flowering processes with highly unpredictable timing and intensity of flowering, unreliable fruit yield and, for horticultural varieties, economic consequences (Petri and Herter 2002; Darbyshire, Webb et al. 2011; Marafon, Citadin et al. 2011). In many of the world's traditional fruit growing regions, marginal locations have become especially vulnerable to the deleterious conditions predicted to occur under projected climate change scenarios (Luedeling, Gebauer et al. 2009), viz. warmer winters with increasing likelihood of inadequate chill exposure, more frequent extreme summer-heat events, and drier winters with increased significance of breaking rains following extended periods of drought.

This investigation focuses on the impacts of a marginal, Mediterranean-type climate on the flowering phenology of *Pyrus* species growing at the Waite Arboretum in Adelaide,



South Australia. The main objective was to determine the principal climatic drivers, and specific triggers, of flowering; in particular, the responses to minimum winter temperature, rainfall in the months preceding the flowering season, and the extremes of summer temperatures and extended drought. The data set also provided a unique opportunity to compare similarities and differences in phenology of individual trees and species growing under identical climatic conditions, and to appraise the impacts of a southern climate on a deciduous, horticultural species from the northern hemisphere.



**Fig. 1** A trio of *Pyrus amygdaliformis* trees flowering in the Waite Arboretum. Each year trees flower in succession: flowering of tree front left begins about one week in advance of tree front right, which in turn comes into flower about one week ahead of the taller tree to the rear, centre (flowering just beginning in this photograph).

## Materials and Methods

### *Site location and history*

The Waite Arboretum of the University of Adelaide in South Australia is situated in the foothills five kilometers south of the city ( $34^{\circ} 58'S$ ;  $138^{\circ} 38'E$ ). The site is generally representative of a more extensive region economically dependent upon a competitive horticultural industry. The pear trees which are the focus of the study are all situated within the grounds of the arboretum and experience the same ambient climatic conditions. They have not received supplementary water. Each tree has been the subject of ongoing phenological observation since its planting, providing a data set ideally suited for the study of the phenological response of this horticultural genus to the local climate.

### *Phenological observations*

Long-term phenological records provide a useful means of determining the biological response to climate variations at specific sites (Sparks and Carey 1995). At higher latitudes where the available envelope of suitable conditions for progress from budding to fruiting has well defined limits, anthesis is synchronized and flowering is a well timed, predictable and eagerly anticipated event (Martínez-Valero, Melgarejo et al. 2001; Meier 2001; Valentini, Me et al. 2001; García-Carbonell, Yagüe et al. 2002; Salazar, Melgarejo et al. 2006). In a region where seasonal conditions are much less clearly defined, there are practical difficulties in capturing the onset of flowering, as it often commences before annual recording schedules begin. The early stages of flowering may also extend for a period of weeks before the commencement of full flowering. Hence the reliable capture of first flowering events requires regular, ongoing observations over many months, and they may not be a good indication of the timing of full, or peak, flowering occurrences.

### *The data set*

The flowering data for this study comprises 44 years of recorded observations of *Pyrus* phenology documented at the one site between 1967 and 2010. A total of 112 trees

comprise 21 named species/varieties/cultivars, two named hybrids and 11 trees as unnamed species. Observations have not been made in all years, and within years there are many gaps and some inconsistencies, although relatively few for such a long series. Recordings for individual trees range from comprehensive – covering the full flowering phenophase in most years since planting (< 25% of the trees) to insignificant – 1 to <10 records in total (> 25% of the trees).

Early flowering stages recorded in the data set were highly variable in timing and gave little indication of the timing and intensity of peak flowering. This phenophase was the stage most commonly missing from the records. Such irregularities in date and duration validate the choice of peak flowering as the more reliable, although still highly variable, metric. Furthermore, for those trees and species which did not have recorded evidence of unseasonal flowering episodes, such events cannot be ruled out; it may have been that these events occurred but were not observed and/or recorded. Despite deficiencies, this data set satisfied a unique opportunity to investigate, not only the flowering phenology of an introduced, deciduous species, but also to compare similarities and differences of individual trees and species subjected to the same ambient climatic conditions.

Climatic data were obtained from the Australian Bureau of Meteorology <http://www.bom.gov.au>. As no one station provided the required data, temperatures were Adelaide station at Kent Town, situated five kilometers north of the arboretum, and rainfall from the Glen Osmond station approximately one kilometre east (see also Appendix A4). Mean minimum temperature is 12.2<sup>0</sup>C, with the lowest recorded minima of -0.4<sup>0</sup>C. Annual precipitation has a considerable range of 706mm, from 304mm to 1010mm, with mean of 628.3mm (Appendix A.4).

### *Analysis*

To avoid the uncertainties inherent in first flowering records, this investigation focused on the timing of peak flowering. This represents the period when an individual tree displayed maximum flowering for any one year, and is not to be confused with full canopy

flowering which, for some trees, may only be achieved in occasional years. Peak flowering day (PFD) was calculated as the number of days from the winter solstice; PFD one  $\equiv$  22<sup>nd</sup> June. Where the stage of peak flowering was not specifically recorded, it was often relatively easy to interpolate and gauge PFD with a high degree of confidence, given detailed flowering observations immediately prior to, and following, this event. This calculation could not be applied to date first flowering. The main analysis was based on 25 trees which were represented by  $\geq 12$  peak flowering data entries. Each tree was identified by a unique identity number.

As chill-sensitive species such as pears (Luedeling, Gebauer et al. 2009; Tanino, Kalcsits et al. 2010) are highly responsive to increases in daily minimum temperatures during warmer winters (Heide and Prestrud 2005), the lowest mean minimum monthly temperature for the calendar year of observation ( $T_m$ ) was chosen as the most appropriate temperature indicator (Luedeling, Gebauer et al. 2009; Tanino, Kalcsits et al. 2010; Panchen, Primack et al. 2012). Because flowering was so variable from year to year and from species to species, it was difficult to find an appropriate standard rainfall measure which necessarily avoided a statistically circular argument (Primack, Ibáñez et al. 2009). The relative impacts of various periods of rainfall prior to the flowering season were trialled to obtain the most appropriate indicator for the main analysis.

Boxplots and One-way ANOVA of peak flowering days for the 25 trees, with species as a factor, along with Tukey HSD post-hoc multiple comparisons of means, provide an initial overview of peak flowering phenology of species and individual trees. Multiple linear regression was then used to investigate the relative significances of the month in which this event occurred ( $T_m$ ), and the total precipitation in the six monthly period from November to April preceding flowering. Where assumptions of normality and linearity were violated, as with data representing trees and species prone to multiple, bimodal flowering and summer flowering events, these were eliminated from the regression analysis. Following this main analysis, a further assessment of these phenomena was based on the flowering regime of individual trees which had been thus affected by

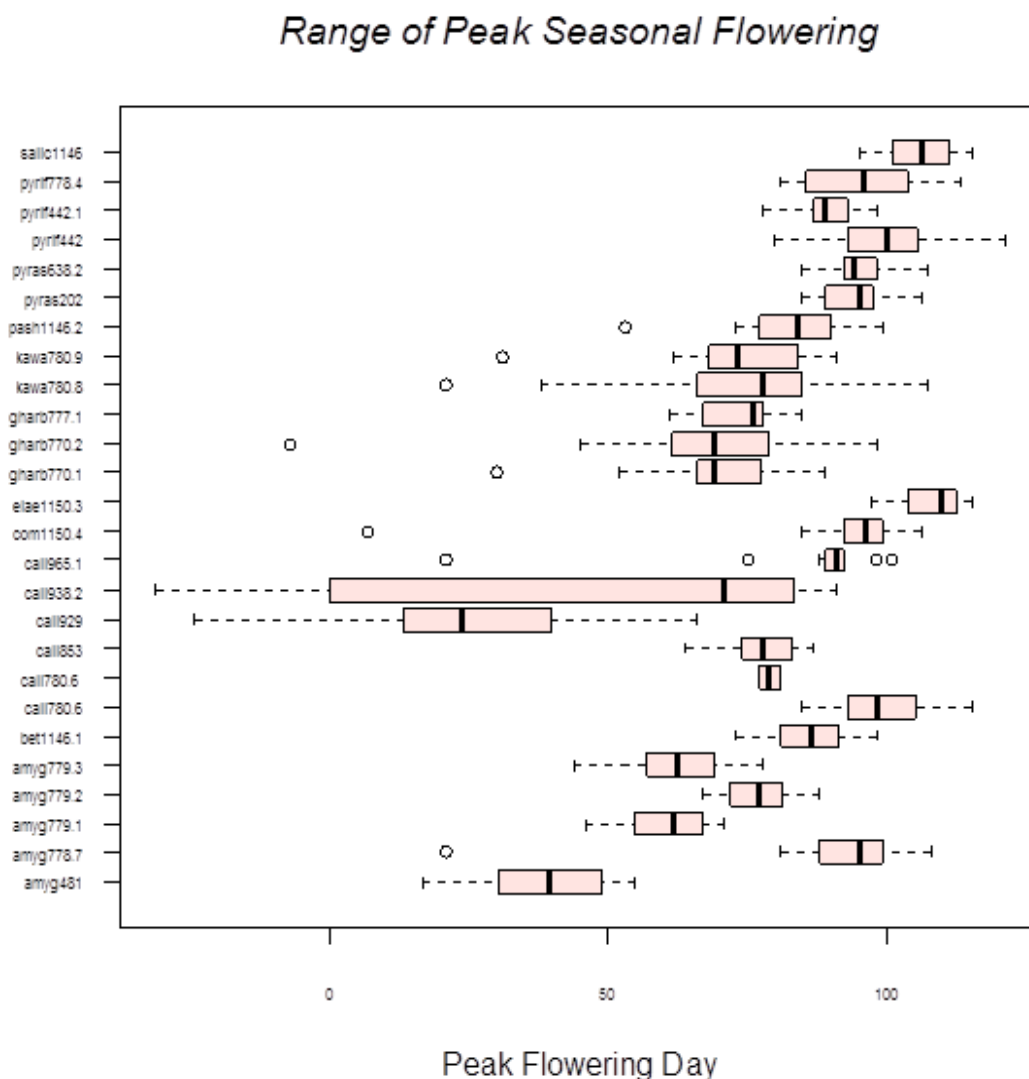
exposure to severe drought conditions and periods of extreme summer maximum temperatures.

## **Results:**

### ***Overview of flowering phenology***

Flowering of *Pyrus* at the Waite Arboretum typically begins in winter, and continues into spring, but has been recorded in all months other than December. This does not necessarily mean that flowering did not occur during this month, merely that it was not recorded: a reasonable possibility as December is also the month when observations are least likely to be carried out. Bi-modal and multiple flowering events were relatively common. Bimodal here refers to one continually documented period of flowering but with two separate mass flowering episodes, one usually more intense than the other. Multiple flowering events indicate flowering episodes which occurred in any one year, but which were separated by a period when no flowers were recorded as present. Some trees and species did not flower at all in some years (i.e. were actually recorded as having no flowers present).

The boxplot (Fig. 2) well illustrates the diverse nature of the flowering phenophase of *Pyrus* trees growing at the Waite Arboretum. Recorded PFD extended from day -31 to day 121, a period of 21 weeks and 5 days, recorded over a period of 6 months (May to October) and spanned three seasons (autumn, winter and spring). This range was evident both within and among the constituent species. One-way ANOVA confirmed a highly significant species effect on PFD ( $P < 0.001$ ).



**Fig. 2** Peak flowering day (PFD) for 11 species (25 individual trees). PFD zero represents 21st June; PDF 50: 10th August; PDF 100: 29th September. Across species, recorded PFD extended from -31 to 121, a period of 21 weeks and 5 days, spanning 6 months (May to October inclusive), and three seasons (autumn, winter and spring). The double occurrence of *P. calleryana* tree # 780.6 (call780.6) indicates bimodal and multiple flowering events for this tree. Species codes: amy: *P. amygdaliformis*; bet: *P. betulifolia*; call: *P. calleryana*; com: *P. communis*; elae: *P. elaeagrifolia*; gharb: *P. gharbiana*; kawa: *P. kawakamii*; pash: *P. pashia*; pyras: *P. pyraeaster*; pyrif: *P. pyrifolia*; salic: *P. salicifolia*.

Tukey HSD (post-hoc multiple comparisons of means; 95% family-wise confidence level) identified significant differences between species as summarized in Table 1. Records for *P. calleryana* tree #938.2 extended from day 21<sup>st</sup> May (PFD -31) to 20 September (PFD 91) represent the longest recorded flowering period of 122 days, spanning 5 months and 3 seasons. This contrasts with *P. salicifolia* 3 weeks (PFD 95 to 115) and *P. elaeagnifolia* 2 weeks and 5 days (PFD 97 to 115), both of which flowered late in the season from the end of September into October.

**Table 1** Summary of Tukey HSD post-hoc multiple comparisons of means for species groups represented by more than one tree; 95% family-wise confidence level. Species effect is not inclusive of all species combinations.

	<i>P.call</i>	<i>P.comm</i>	<i>P.gharb</i>	<i>P.kawa</i>	<i>P.pyras</i>	<i>P.pyrif</i>
<i>P.amygdaliformis</i>	n.s.	< 0.001	n.s.	n.s.	< 0.001	< 0.001
<i>P.calleryana</i>		< 0.001	n.s.	n.s.	< 0.001	< 0.001
<i>P.communis</i>			< 0.001	0.007	n.s.	n.s.
<i>P.gharbiana</i>				n.s.	< 0.001	< 0.001
<i>P.kawakamii</i>					0.001	< 0.001
<i>P.pyraster</i>						n.s.

Some species exhibited a greater plasticity (see page 151) in their flowering response than others. *P. pyraster* and *P. pyrifolia* ( $P=1.000$ ) showed greatest similarity, uniformity and consistency in their range of peak seasonal flowering, while *P. gharbiana* and *P. kawakamii* ( $P=0.999$ ), although similar, had a tendency to greater plasticity in their response. In contrast, *P. amygdaliformis* and *P. calleryana* provided evidence of much greater diversity in flowering time, but whereas individual trees of *P. amygdaliformis* showed a similar pattern (although in different time frames), *P. calleryana* trees were characterized by much greater among-tree variation (*P. calleryana* #853: PFD spanned 23 days; *P. calleryana* #938B: of PFD spanned 122 days). The latest flowering *P. salicifolia*

and *P. elaeagrifolia* each reveal a brief range of PFD, although each was represented by only one tree.

The boxplot also suggests a late season threshold for flowering, such that the later the median PFD, the shorter the overall peak flowering period. This link between flowering period and median was tested and confirmed: the regression of median PFD on range of PFD (with outliers identified in the boxplot removed from the analysis) showed that, on average, the median flowering date occurred almost four days later for each reduction in the overall peak flowering period by 10 days ( $\beta = -0.384$ ;  $P = 0.016$ ).

Within species, individual trees also exhibited a variety of flowering responses. Greatest within species variation was detected in *P. amygdaliformis* and *P. calleryana*. The four individual trees of *P. amygdaliformis* showed minimal temporal overlap which contributed to the broad range of variation exhibited by this species. Tree # 481 was consistently early flowering in winter compared with tree # 778.7 which was consistently late coming into full flower (September, October), although an early flowering event was recorded. Extreme differences, with no evidence of similarity, are apparent among the five representative trees of *P. calleryana*. Extremes range from early (#938.2 and #929) to late (#780.6) and from consistently concise flowering events (tree # 780.6) to broadest range (for #938.2) to tree # 965.1 with a short concise flowering pattern but a greater tendency than other trees for bimodal events. In contrast, trees representing *P. gharbiana*, *P. kawakamii*, *P. pyraster* and *P. pyrifolia* have a generally synchronous within species flowering record; the PFD of the former two species occurring mid-season but with a greater temporal span and a tendency for an early response; the latter flowering consistently later in the season, for a briefer period with greater concision.

The high variability in flowering phenology for all species combined incorporates a shift to earlier flowering of about 8 ½ days over the period from 1980 to 2010. A simple linear regression of PFD on Year indicates a general advance of PFD of between 1.4 and 4.2 days per decade ( $\beta = -0.2835 \pm 0.1394$ ,  $P = 0.0426$ ). This shift coincides with the period of warming which has occurred simultaneously over the same period (Appendix A.1).



***Phenological response to climatic drivers***

The genus (i.e.) showed a very highly significant overall response to mean minimum monthly temperature ( $T_m$ ) ( $\beta = -7.13 \pm 1.211$ ;  $P < 0.001$ ), an advance of around 7 days for each  $^{\circ}\text{C}$  rise in temperature. The month in which this minimum temperature occurred was also a highly significant indicator of PFD ( $\beta = 11.69 \pm 1.84$ ;  $P < 0.001$ ). This represents an advance of around 11 ½ days for each month of the year earlier in which  $T_m$  occurs; e.g. when  $T_m$  occurred in July, PFD was, on average, 11 ½ days earlier than when  $T_m$  was recorded in August.

Measures of precipitation for periods prior to the month of flowering produced highly significant, but invalid, effects, as they incorporated a statistically circular argument (Primack, Ibáñez et al. 2009). The impact of total rainfall in the 12 months preceding the month of peak flowering was insignificant. However, some indication of a trend to earlier flowering of around 5 days per 100 mm rainfall was found for total rainfall during the 6 months from November to April preceding flowering ( $\beta = -0.047 \pm 0.026$ ;  $P = 0.068$ ). This was the measure chosen for the multiple regression analysis for combined and individual species (Table 2).

Each species displayed a unique response to the three climatic predictors (Table 2), often representing a considerable departure from the overall response of the genus. PFD of *P. calleryana* occurred approximately 8 days earlier for each  $^{\circ}\text{C}$  increase in  $T_m$ , while PFD was delayed by about 3 days for *P. pyrifolia* ( $\beta = 3.03 \pm 1.66$ ;  $P = 0.074$ ) and almost 3½ days for *P. salicifolia* ( $\beta = 3.42 \pm 1.32$ ;  $P = 0.019$ ) for the same  $^{\circ}\text{C}$  increase in minimum temperature.



**Table 2** Summary of multiple regression analysis for combined *Pyrus* spp. represented in the boxplot (Fig. 2) plus the subset of species which satisfy the assumptions of normality and linearity. Data for 2008 have been removed from *P. communis* 1150D (normality assumptions violated with 2008 data). *P. communis* and *P. salicifolia* are each represented by only one tree. Tmin: lowest mean monthly minimum for the year ; ppt per mth: total precipitation for the 6 months preceding the month of flowering; ppt 6mths: total precipitation for the 6 months from November to April preceding flowering; ppt 12mths: total precipitation for the 12 months preceding the month of flowering. Total rainfall for the preceding 6 months from November to April was the measure chosen for the analysis of individual species. Significance codes: 0 ‘\*\*\*’ for  $P<0.001$ ; ‘\*\*’ for  $P<0.01$ ; ‘\*’ for  $P<0.05$ ; ‘.’ For  $P<0.1$ .

PFD	Year	Tmin	Month of Tmin	ppt 6mths	ppt 12mths
<i>Pyrus</i> spp.	-0.239 .	<b>-6.99 ***</b>	<b>12.16 ***</b>	-	0.002
<i>Pyrus</i> spp.	<b>-0.256 *</b>	<b>-7.13 ***</b>	<b>11.69 ***</b>	-0.026	-
<i>P amygdaliformis</i> sp.	0.160	<b>-6.61 ***</b>	<b>10.85 ***</b>	-0.029	-
<i>P. calleryana</i> sp.	-0.174	<b>-8.19 **</b>	<b>20.27 ***</b>	0.018	-
<i>P. communis</i> 1150D	-0.091	0.921	2.59	-0.043 .	-
<i>P pyraster</i> sp.**	-0.121	-0.160	2.92	-0.021	-
<i>P. pyrifolia</i> sp.	-0.219	3.028 .	1.44	<b>-0.064 *</b>	-
<i>P salicifolia</i> 1146	-0.0226	<b>3.421 *</b>	0.638	-0.0238	-



The responses of *P. amygdaliformis* and *P. calleryana* were most typical of the genus, with highly significant advancement of about one week for each °C rise in minimum temperature, and a negative but non-significant effect for rainfall (NB positive for *P. calleryana*). *P. communis* and *P. pyrastrer* were the only two species under investigation which showed no significant response to Tm, or the month in which it occurred. *P. pyrastrer* showed no significant response to any of the variables. All species other than *P. calleryana* showed a general trend to earlier flowering with higher precipitation, but this effect was significant only for *P. pyrifolia* ( $P=0.021$ ) and for *P. communis* at 10% ( $P=0.065$ ).

### ***Impact of extreme climatic events***

#### *Phenological response to breaking rainfalls*

##### Multiple flowering events 2008

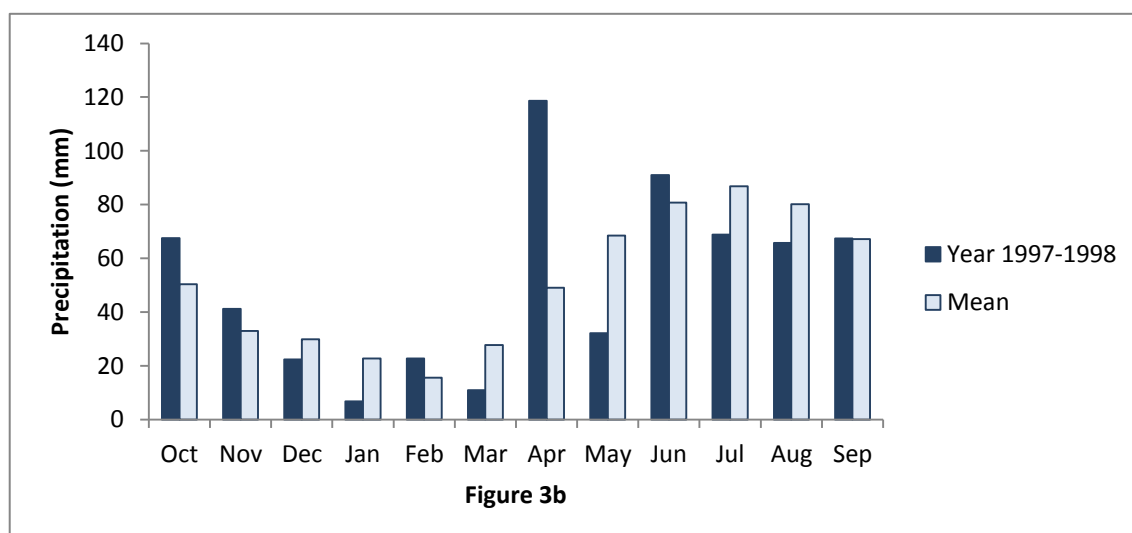
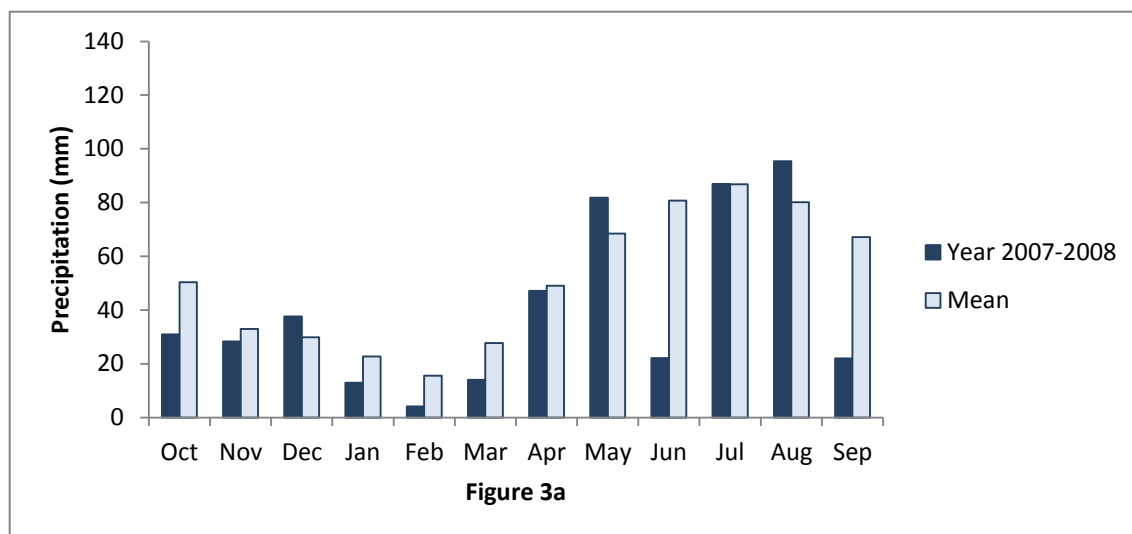
Rains in late April and May of 2008 (Fig. 3a), the driest year, marked the end of a severe drought during which 29 of the preceding 38 months received below average falls; some well below. This drought breaking rain triggered multiple flowering events in 11 trees (Table 3)

### **May flowering**

May flowering events were recorded in 1989, 1998 and 2007 (Table 4), well before the lowest mean monthly minimum temperatures for the year occurred. These were years when rainfalls in April and/or May were well above average. In 1989, breaking rain in May followed four months of well below average falls (total of 42mm from January to April inclusive; mean for this period is 120mm). In 2007, well above average rainfall in April and May followed by below average rainfall in the first three months of the year and in the previous year (total for 2006 was 358mm; yearly mean 628mm<sup>6</sup>) triggered heavy, full flowering in late May. These early, unseasonal responses were observations generally restricted to *P. calleryana*, *P. amygdaliformis* and their named hybrid *P. 'Prescott'*, with one record each for *P. gharbiana* #777A and *P. communis* #477 ( this was not the same tree observed to flower in June 2008 – see Table 3 below).

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<sup>6</sup> Yearly mean calculated for years: 1884-2012: <http://www.bom.gov.au/jsp/ncc/cdio/weatherData>;



**Fig. 3a** Monthly rainfall at Glen Osmond from October 2007 to September 2008. Of the 47mm April monthly total, 34mm fell on or after the 26th of the month. This was followed by above average falls of 81.8mm in May but very low falls in June of 22mm (mean for month 86.9mm). Total rainfall in the months of July (87mm) and August (95.4mm) was above average. **Fig. 3b** Monthly Rainfall at Glen Osmond from October 1997 to September 1998. Above average rainfall in April triggered flowering in May.

**Table 3** Multiple flowering events in 2008. Of the 47mm April monthly total, 34mm fell on or after the 26th of the month. This was followed by above average falls of 81.8mm in May but very low falls in June of 22mm (mean for month 86.9mm). Total rainfall in July (87mm) and August (95.4) was above average (see Fig. 3a).

Tree #	Tree name	Flowering episode 1	Flowering episode 2
202	<i>P. pyraister</i>	25 June-8 July	16 Sep – 7 Oct
770A	<i>P. gharbiana</i>	19 July-12 August	19 Aug – 1 Oct
780H	<i>P. kawakamii</i>	28 June-12 Aug	2 Sept-8 Oct
780J	<i>P. kawakamii</i>	12 July-12 Aug	26 Aug-8 Oct
929	<i>P. calleryana</i>	1 June-12 Aug	19 Aug-23 Sep
932C	P. Prescott	1 June-12 July	12 Aug-16 Sep
938B	<i>P. calleryana</i>	1 June-12 Aug	26 Aug – 14 Oct
938C	<i>P. calleryana</i>	1 June – 2 Sep	9 Sep- 14 Oct
951C	<i>P. calleryana</i> ‘Chanticleer’	10 June-19 Aug	9 Sep-14 Oct
965A	<i>P. calleryana</i> ‘Redspire’	1 July-29 July	2 Sep-14 Oct
1150D	<i>P. communis</i>	23 June-22 July	20 Sep-14 Oct

**Table 4** May flowering events recorded in 1989, 1998 and 2007. With the exception of *P. communis* and *P. gharbiana* in 1989, these events were restricted to two species and their hybrid (*P. 'Prescott'* = *P. amygdaliformis* x *P. calleryana*). Flowering dates were not exceptionally early for *P. calleryana* #292. FB: flowering beginning; FFB: few flowers beginning; HFB: heavy flowering beginning; FFF: few full flowering; HFF: heavy full flowering; vHFF: very heavy full flowering; FE: flowering ending.

Year	Species	Tree	Date	Status
1989	<i>P. calleryana</i>	929	30 May	HFB
1998	<i>P. amygdaliformis</i>	481	21, 28 May	FB
	<i>P. amygdaliformis</i>	778G	28 May	FFF
	<i>P. calleryana</i>	777A	28 May	FFF
	<i>P. calleryana</i>	853	21 May	FFF
	<i>P. calleryana</i>	929	21 May; 28 May	FB; HFF
	<i>P. calleryana</i>	938C	21 May; 28 May	FFF; FF
	<i>P. 'Prescott'</i>	932A	21 May	FE
	<i>P. 'Prescott'</i>	932B	28 May	FE
	<i>P. 'Prescott'</i>	932C	21 May; 28 May	FFF; FE
	<i>P. communis</i>	477	28 May	FFF
	<i>P. gharbiana</i>	777A	28 May	FFF
2007	<i>P. calleryana</i>	929	30 May	vHFF
	<i>P. calleryana</i>	938C	30 May	HFF
	<i>P. calleryana</i>	942A	30 May	HFF
	<i>P. calleryana</i>	942B	30 May	HFF
	<i>P. 'Prescott'</i>	932A	30 May	HFF
	<i>P. 'Prescott'</i>	932B	30 May	HF
	<i>P. 'Prescott'</i>	932C	30 May	FFB

#### *Response to extreme heat events*

Unseasonal flowering episodes were also recorded for some trees during the months of February, March and April (Table 5); in each case following extreme heat events (see



Appendix B for plots of maximum temperatures above 37<sup>0</sup>C) when temperatures reached  $\geq 40^0\text{C}$  in January;  $\geq 40^0\text{C}$  in February. The extent of flowering varied from full to sparse and, despite comparable temperature patterns in 2010 and 1985, no unseasonal flowering occurred in 2010<sup>7</sup>. Although these unusual flowering events occurred in autumn, for expediency, this response will hereafter be referred to as ‘summer’ flowering.

**Table 5** Late summer and early autumn flowering episodes in each case following extreme heat events flowering events. FB: flowering beginning; HFB: heavy flowering beginning; F: flowering; FFF: few full flowering; HFF: heavy full flowering; HFE: heavy flowering ending; FE: flowering ending. *P. korshinskyi* = *P. communis* ssp. *korshinskyi*. *P. korshinskyi* 778.4 and 778.7 were the only trees to be recorded with unseasonal flowering in more than one year.

Tree #	species	Age (years)	year	Feb	March	April
778.3	<i>P. calleryana</i>	5	1983		18:FF; 20:FF	
778.7	<i>P. korshinskyi</i>	4	1983		31: FFE	
461	<i>P. calleryana</i>		1983		31: FFF	
778.4	<i>P. korshinskyi</i>	7	1985			12:FF; 14:FE
778.7	<i>P. korshinskyi</i>	6	1985			12:FF; 14:FE
1150.4	<i>P. communis</i>	16	1985			12:HFF
777.1	<i>P. calleryana</i>	1	1991			18:FF
778.4	<i>P. korshinskyi</i>	23	2001			27:HFE
481	<i>P. amygdaliformis</i>	31	2003		27:FFF	
2901	<i>P. nivalis</i>	6	2007		19:HFB	
2903	<i>P. nivalis</i>	8	2009	18:FB	6:F	
2905	<i>P. calleryana</i>	12	2009		1: FFF	
2907	<i>P. calleryana</i>	12	2009		1: FFF; 6:F	
2908	<i>P. Prescott</i>	10	2009		1: FFF; 6: F	

<sup>7</sup> 2010 was the only known year in which summer flowering was checked and found not to occur.

## Discussion

### *Overview of flowering phenology*

While a shift to earlier flowering occurred for the pear trees growing at the Waite Arboretum, Adelaide, South Australia, a varied range of responses of these trees was evident, both within and between species, and included prolonged, unseasonal and multiple flowering episodes following heavy rainfalls at the end of prolonged dry periods and highly unseasonal flowering from summer into autumn following extreme summer temperatures. Both mean minimum monthly temperature and rainfall were found to have substantial impacts on the flowering phenology of the plantation trees in general, but it was only within the context of time of year and the generally prevailing climatic circumstances that these triggers could be meaningfully assessed. While the present data set was insufficient to accommodate an in-depth study of these phenomena, such interesting findings warrant further investigation.

### *An overview of phenology of individual trees and species*

It has been reported (Challice and Westwood 1973) that many of the *Pyrus* growing in the botanical gardens of Europe and the USA have been wrongly identified, including a high proportion of dubious hybrid origin. While this situation has now possibly been revised, early plantings and seedlings at Waite may have been a misidentified. For this reason, groupings according to the more inclusive regional pear-type categories as per Challice and Westwood (1973) and Volk et al. (2006) give further basis for consideration (Table 6).

**Table 6** Regional groups of *Pyrus* species as per Challis and Westwood (1973)

<b>Regional group</b>	<b><i>Pyrus</i> species</b>
European	<i>P. communis</i> sensu lato; <i>P. communis</i> var. <i>pyraster</i> ; <i>P. nivalis</i> ;
West Asian	<i>P. amygdaliformis</i> ; <i>P. elaeagrifolia</i> ; <i>P. syriaca</i>
Larger fruited Asian	<i>P. ussuriensis</i> ; <i>P. pyrifolia</i> ; <i>P. pashia</i> ;
Asian pea pears	<i>P. betulifolia</i> ; <i>P. calleryana</i> ; <i>P. kawakamii</i> syn. <i>P. calleryana</i> var. <i>calleryana</i>
North African	<i>P. mamorensis</i> ; <i>P. gharbiana</i>

**Table 7** Significant differences (D) in flowering phenology of species according to region of origin. P-values < 0.05 from Tukey HSD. D: one instance of difference; DD: two instances of difference, etc.

	Europe	West Asia	Larger Asian	Asian pea
Europe				
West Asia	DD			
Larger Asian		D		
Asian pea	DDDD		DD	
North Africa	DD		D	

In agreement with recent naming reclassifications (Challice and Westwood 1973; Volk, Richards et al. 2006), no significant differences were found between *P. communis* and *P. pyraster* (reclassified as *P. communis* var. *pyraster*) or between *P. calleryana* and *P. kawakamii* (syn. *Pyrus calleryana* var. *calleryana*). Similarities and differences are evident across regional ecotypes (Challice and Westwood 1973; Myking and Heide 1995) (Table 7), but no significant differences in flowering phenology were found between pears of European origin and Larger fruited Asian pears; between West Asian and Asian pea pears; or between North African and West Asian and Asian pea pears. There appears to be, therefore, a phylogenetic basis determining responses at this level.

### ***Phenological response to climatic drivers***

#### ***Response to temperature and rainfall***

Floral development and anthesis do not usually follow directly from the initial stages of bud differentiation but are withheld until *conditions conducive to flowering* prevail (Opler, Frankie et al. 1976; Battey 2000). In the variable Mediterranean-type climate of Southern South Australia, *Pyrus* flowering was recorded in all months other than December. Once again, this does not imply that flowering has not occurred in December, just that it has not been recorded. Hence the need for year round observations to obtain a reliable data set.

For most trees and species, in years of sufficient rainfall, regular seasonal flowering is under the control of winter temperature. Flowering generally peaked about a month after the coldest mean minimum monthly temperature ( $T_m$ ). However, the colder the  $T_m$ , the later peak flowering occurs. Thus, when temperatures began to increase following the coldest month, peak flowering is triggered: the less cold the temperature, the earlier the flowering. *P. pyrifolia* and *P. salicifolia* were exceptions. This response to temperature may be construed as an alternative vernalization requirement for a longer period of warmer weather needed to compensate for lack of cold (Yu, Luedeling et al. 2010; Okie and Blackburn 2011). As Okie and Blackburn (2011) demonstrate for peach cultivars (*Prunus persica* (L), time and heat accumulation required for bud break increase in a curvilinear relationship as chilling exposure decreases, at times resulting in an extended and asymmetrical onset of flowering. crops which retain a residual effect of dormancy as a result of insufficient chilling display symptoms such as deficient flowering, fruit set and uneven foliation (De Melo-Abreu, Silva et al. 2005; Marafon, Citadin et al. 2011).

If a given species flowers as much as two months apart in different years, photoperiod and thermal accumulation are deemed inadequate to explain the observed phenology, and rainfall may act as the important trigger and spacing mechanism (Opler, Frankie et al. 1976). While no convincing species effect of rainfall was found in the current investigation, individual trees of the species *P. amygdaliformis*, *P. calleryana* and *P. communis* responded to opening rainfalls following extended periods of dryness. However, when progression to flowering is dependent on highly unpredictable rainfall events, the timing of this phenophase will be likewise unpredictable. and without the more reliable temperature cues, there is a good possibility that plants may mis-time their response and flower at an inopportune time.

### ***Impact of extreme climatic events***

#### *Phenological response to breaking rainfalls*

*Pyrus* in South Australia responded to rainfall in a similar manner as did the native trees in the tropical conditions of Costa Rica (Opler, Frankie et al. 1976). In both cases, it was rainfall in the latter half of the dry season which had an overriding influence on flowering.

These dissimilar species, growing in widely different locations, responded in a similar fashion: some species failed to flower at all in some years, and countered with multiple flowering episodes in other years.

The nature of multiple flowering events triggered by drought relieving, or drought breaking, rains appears likely to depend on the severity of the drought and the pattern of follow-up rainfall. With insufficient water, early flowering attempts are aborted, often to be re-instigated later in the year following further good falls. Of relevance to this study, the months leading up to May flowering episodes in 1998 and 2007 (Fig. 3b) were not as dry as those in 2008, and exceptionally high falls in April were apparently sufficient to support remnant flowering through the season and, in some cases, to initiate a second mass blooming episode.

While flowering episodes for pear in 1998, 2007, 2008 were neither as well synchronized nor as brief as reported by Opler et al. (1976) for the native species of Costa Rica, this response seems well established in some *Pyrus* species. The apparent diversity of species is reduced to two, however, when considering *P. 'Prescott'* is a cross of *P. amygdaliformis* and *P. calleryana*, *P. kawakamii* has been re-identified as a variety of *P. calleryana*, and the European *P. communis* var.(ssp) *pyraster* is associated with the West Asian fruiting *P. amygdaliformis* (Challice and Westwood 1973).

#### *Response to extreme heat events*

*Pyrus amygdaliformis* and *P. calleryana* were also the primary species exhibiting the flowering anomaly triggered by extreme heat events at the end of summer (Appendix B). It is of interest to note that summer flowering has not been recorded in any two consecutive years although, again, it may have occurred. When compared with the other charts, the pattern of temperature in the summer of 2010 seems to meet requirements for

this flowering phenomenon. However, flowering did not ensue<sup>8</sup>, making it difficult to reach conclusion as to the precise temperature trigger for flowering to occur.



**Fig. 4** Flowers and mature fruit competing for space and resources on branch of *P. calleryana* #2907 following an extended period of extreme heat in January and February (see Appendix A.2). Such unseasonal flowering compromises fruit quality from the previous year's flowering and the quantity of fruit in the following spring. Photo taken at Waite Arboretum 7<sup>th</sup> March 2009.

In 2009, I personally noted that this flowering response was mainly limited to younger trees. Some of the more mature individuals showed signs of early bud development, often with young terminal leaves shooting, but these trees did not produce flowers. Age may be a factor as most trees were 12 years of age or under at the time of the flowering event,

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<sup>8</sup> The only recorded year in which trees were observed and found not to be flowering during the summer months.

although trees of 23 and 31 years of age were recorded flowering in March and April (Table 5). Summer flowering also coincided with ripening of the previous season's fruit, and at least one tree in 2009 presented all stages of development from bud initiation to the presence of mature fruit (Fig. 4).

Plants exposed to extreme temperatures rely on a rapidly responding heat shock mechanism for protection (Key, Lin et al. 1981) but, whereas a temperature shift from 28-30°C to 40°C leads to a rapid protective shift in the pattern of protein synthesis, at temperatures above 40°C heat shock synthesis is more transient (Ferguson, Lurie et al. 1994). This allows the devernalisation and subsequent flowering events (Balasubramanian, Sureshkumar et al. 2006; Halaly, Pang et al. 2008; Campoy, Ruiz et al. 2011). While seemingly unrelated, temperature responsive, heat shock proteins are thought to be related to proteins synthesized at low temperatures during chilling (Key, Lin et al. 1981; Guy 1990; Tamura, Tanabe et al. 1998)

There are multiple references to extreme heat exposure effectively providing an alternative de-vernalization mechanism leading to unseasonable flowering (Ferguson, Lurie et al. 1994; Tamura, Tanabe et al. 1998; Tamura, Tanabe et al. 2001; Balasubramanian, Sureshkumar et al. 2006). While pears appear especially vulnerable, peach cultivars with low chilling requirements can be stimulated to flower in autumn after exposure to elevated temperatures (Erez, Faust et al. 1998; Campoy, Ruiz et al. 2011). Likewise, dormancy release in grape buds can be induced by an external heat shock stimulus, with bud break following in about four weeks (Halaly, Pang et al. 2008). These studies show that there is only a very small window in which elevated temperatures are conducive to the development of flowering. While it is not possible to gain a clear understanding of the particular response of pears in this study, the phenomenon has important economic consequences and merits further investigation.

## Conclusion

### *Impacts of marginal growing conditions of a southern climate on a deciduous, northern-hemisphere adapted, horticultural species*

*Pyrus*, an introduced, deciduous, horticultural genus responds to the climatic extremes encountered in southern South Australia with extreme variation in flowering phenology. Harding et al. (1976), in Kent, England, made comment on the range of full flowering dates of one particular early flowering apple; a spread of 39 days from 8 April to 17 May. This pales in comparison with the spread of 122 days for one *P. calleryana* tree growing at Waite. Often subject to multiple triggers in the course of a 12 month period, particular trees show a confused and erratic phenology at the expense of quality and quantity of the fruit crop.

Response to extreme events cannot be predicted according to the general response to minimum temperature, and some species, or groups of species, are more susceptible than others. Trees of the Asian small-fruited pear *P. calleryana*, including the evergreen *P. calleryana* var. *calleryana* (*P. kawakamii*), show a more flexible range of responses than those of any other documented species. In contrast, the West Asian species, *P. salicifolia* and *P. elaeagrifolia* feature a reliably concise flowering period late in the flowering season, seemingly little affected by the anomalies of the climate. Their phenology may thus be influenced, at least to some extent, by photoperiod (Opler, Frankie et al. 1976). No larger-fruited Asian pear trees and/or species featured in any of the unseasonal events.

This analysis clearly shows that the various species and cultivars have their own specific heat requirements for dormancy release and flowering, with constraints uniquely influenced by genotype. Evergreen varieties of two other woody deciduous species, hazelnut *Corylus avellana* (Thompson, Smith et al. 1985) and peach *Prunus persica* (Rodriguez, Sherman et al. 1994) share a single-deletion mutation affecting multiple genes, which has arisen independently, and on a number of occasions, in both species (Thompson, Smith et al. 1985; Bielenberg, Wang et al. 2004). Cultivars exhibiting this trait fail to respond appropriately to dormancy-inducing conditions and present a wide



range of behaviours relating to time of flower initiation and bloom. While they may be maladapted to withstand the cold of northern winters, hazelnut cultivars which are heterozygous for the non-dormant gene, have established in Turkey and southern Europe (Thompson, Smith et al. 1985).

### ***Likely impacts of climate change***

Plant responses to warming are complex, species specific and regionally dependent (Battey 2000; Yu, Luedeling et al. 2010; Darbyshire, Webb et al. 2011; Okie and Blackburn 2011). Deciduous fruit trees are among those plants most likely to be affected by ongoing climate change (Luedeling, Gebauer et al. 2009; Campoy, Ruiz et al. 2011; Darbyshire, Webb et al. 2011), with projected temperature trends likely to impact on flowering and subsequent fruit production in multiple ways. Under warmer winters, which do not satisfy chilling requirements of vernalization, flowering will be dependent upon the more unreliable alternatives. In marginal areas where such risk is already high, fruit production is susceptible to even slight warming trends, and the economic sustainability of horticultural fruit crops is of particular concern (Luedeling, Gebauer et al. 2009; Darbyshire, Webb et al. 2011). Increased prevalence of extreme heat waves in summer will exacerbate these impacts. Observations of one tree in 2009 showed all stages of development were in process simultaneously, summer flowering coincidental with ripening of the previous season's fruit (see photograph page 146). Economically, such unseasonal responses are highly undesirable because, although fruit set may be accomplished, the resulting fruit is not of commercial quality (Erez, Faust et al. 1998; Campoy, Ruiz et al. 2011). It also jeopardizes the quality and quantity of the crop in the following spring.

Possibly of greatest concern to growers are the early flowering attempts following breaking rains associated with exceptionally dry periods. With rainfall in south-eastern Australia predicted to shift from predominantly winter to more frequent summer events and extreme droughts, these changing patterns can be expected to have greater impact on phenology than directional trends in the amount of precipitation. Field observations show that if early flowering does not proceed to fruiting, or early fruits are aborted, a second,

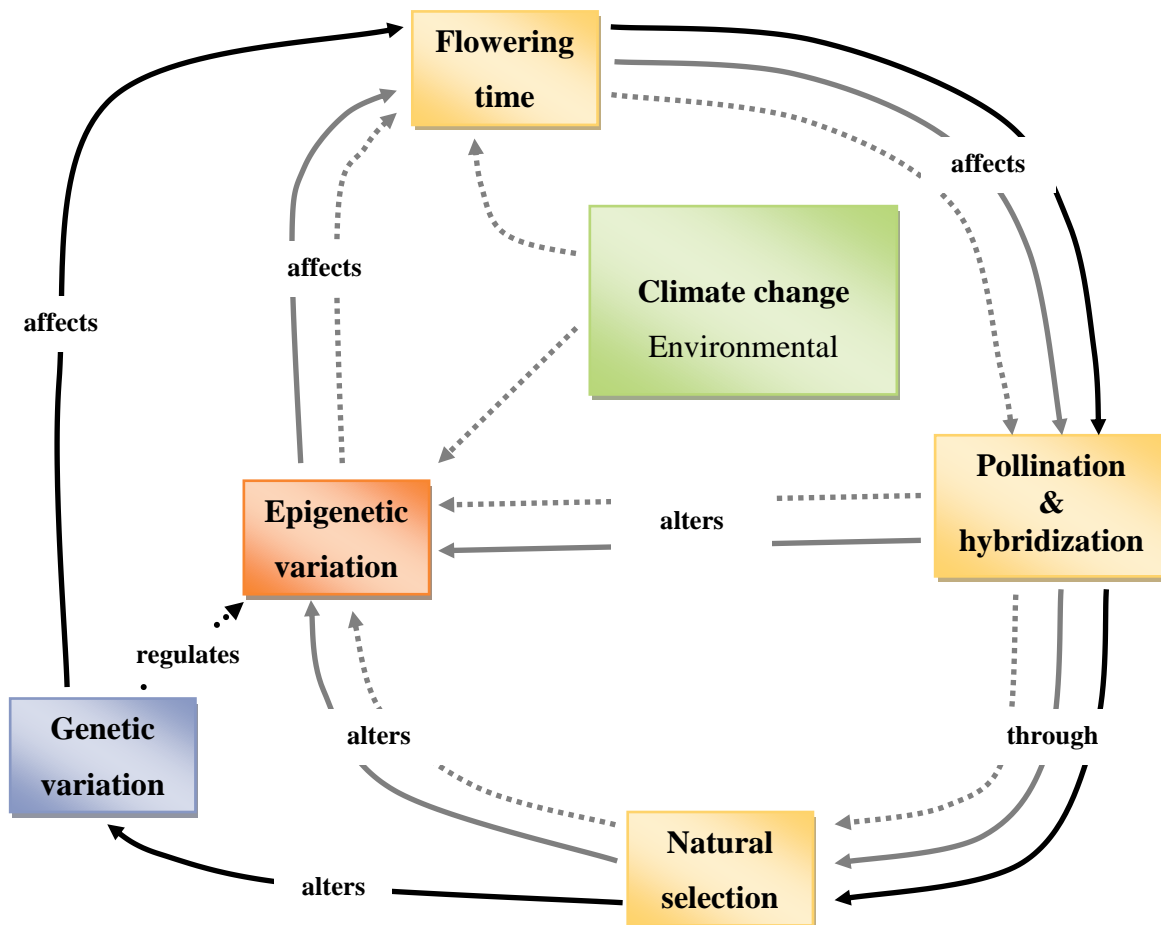
later flowering episode is likely to occur, with reduced crop potential. Climate and weather are key determinants of successful fruit production (Darbyshire, Webb et al. 2011). In the marginal horticultural regions of South Australia, as elsewhere, the greatest impacts of climate change on *Pyrus* horticultural species will be the economic consequences of unreliable fruit yield. However, viability may be enhanced by prudent selection of genotype and appropriate irrigation practices, in particular, the replenishment of soil moisture during extended dry periods and in the months preceding the flowering season to avert early seasonal flowering attempts.

## **Chapter 5 Evolutionary impacts**

While the effects of climate change on phenology are readily apparent, it is less clear how they should be interpreted: are observed phenological shifts signs of ecological disruption, or are they an indication that species are adapting to their changing world? Ongoing fitness in plants entails well-balanced trade-offs: shifts need to be sufficient to track the changing climate while maintaining synchrony with pollinating and seed dispersal agents. Climatically advantageous shifts may compromise timing with specialist pollinators or phenologies of other, closely-related inter-fertile species (de Groot, Ketner et al. 1995; Primack and Miller-Rushing 2012). Fitness also requires successful progress from flowering to fruit maturation, through the avoidance of frost damage by early flowering individuals and unseasonal flowering initiated by breaking rains or extreme summer heat.

Variable flowering times reflect levels of phenotypic plasticity, i.e. the ability of a single genotype to alter its phenotype in response to prevailing environmental conditions (Nussey, Postma et al. 2005). This is an important fitness mechanism enabling reproductive success under the broad range of normally experienced climatic conditions. It is the underlying mechanism regulating current shifts to earlier flowering; it is not indicative of microevolutionary adaptation to a changing climate (Gienapp, Teplitsky et al. 2008; Visser 2008). The effective extent of this plasticity is not known beyond the bounds of recently experienced climatic extremes, and a current concern regards what will happen if, and when, the limits of plasticity are exceeded. An associated concern is the consequential low resilience of small vulnerable populations with depleted levels of variation resulting from bottlenecks and inbreeding (Grant 1981; Arnold 1997).

**Fig. 1** Flow-diagram based on Bossdorf, Richards et al. (2008); epigenetic change  $\Rightarrow$  flowering time  $\Rightarrow$  hybridization  $\Rightarrow$  epigenetic change (Rapp and Wendel 2005) Epigenetic variation (grey lines), unlike genetic variation (black lines), may be altered directly by ecological interactions, and therefore provide an additional, accelerated pathway for evolutionary change. Environmental changes enhanced by climate change are represented by grey dotted lines.



### Genetic variation and constraints

The evolutionary process, by which natural selection acts on phenotypes predetermined by DNA sequence variation, is generally considered too slow to keep pace with the current pace of global warming (Rapp and Wendel 2005; Visser 2008; Richards, Bossdorf et al. 2010) (Fig. 1 black lines). However, successful species can benefit from various other processes promoting phenotypic variation. Flowering time may be particularly responsive to selection when periods of non-overlap in flowering time lead to temporal assortative mating (Fox 2003; Weis and Kossler 2004), inflating genetic variance (Franks, Sim et al. 2007) and promoting local adaptation (Weis and Kossler 2004). A rapid evolutionary response to drought avoidance by the annual, *Brassica rapa*, also represents temporal changes in gene frequencies (Franks, Sim et al. 2007; Franks and Weis 2008). However, these serve to diminish the phenotypes in the population, and under sustained and rapid climate change, this process may deplete genetic variation faster than it can be replenished by mutation.

Epigenetic modifications provide an additional, direct and sensitive response to environmental stimuli, increasing the range of phenotypes independently of DNA sequence changes (Gutierrez-Marcos and Dickinson 2012). Ensuing differences in gene expression can have wide-ranging consequences which persist down generations (Rapp and Wendel 2005; Salmon, Ainouche et al. 2005; Grant-Downton and Dickinson 2006; Richards, Bossdorf et al. 2010). This allows the fittest phenotypes to remain stable for sufficient periods to eventually benefit from more permanent evolutionary processes (Pigliucci 1996; Rapp and Wendel 2005; Salmon, Ainouche et al. 2005; Bossdorf, Richards et al. 2008). This dual system of inheritance thus provides a rapid, although potentially unstable, epigenetic route short-circuiting the slower, but more stable, mutation-dependent route (Bossdorf, Richards et al. 2008; Richards, Bossdorf et al. 2010) (Fig. 1: solid grey and black lines). Through these processes, the environmental conditions which impact on the fitness of organisms also impact on the adaptive mechanisms which beget that fitness.

Epigenetic processes are common in plants (Rapp and Wendel 2005; Chinnusamy and Zhu 2009) and integral with several types of environmentally induced plasticity, including the regulation of flowering time by the process of vernalization (Grant-Downton and Dickinson 2006; Bossdorf, Richards et al. 2008; Campoy, Ruiz et al. 2011) (Fig. 1: solid grey lines). They are also triggered by hybridization (Rapp and Wendel 2005; Salmon, Ainouche et al. 2005) (Fig. 1).

Hybridization is a well known source of novel genotypes which occasionally may prove fitter than one or both of the parental lineages (Stebbins 1959; Arnold 1997; Rieseberg 1997; Barton 2001). This may be particularly so when fitness has been severely compromised by inbreeding. By further contributing additional phenotypes through epigenetic processes, hybridization enhances the retention of genetic information which may otherwise be lost when lineages succumb to the combined stresses of climate and habitat clearance (Kimball, Campbell et al. 2008).

While biologists have long held opposing views as to the benefits of hybridization in relation to the fitness of parental species (Allendorf, Leary et al. 2001), arguments against the value of hybrids tend to weaken as rapid climate change threatens the long-term survival of vulnerable species. Hybrid speciation leading to the establishment of new evolutionary lineages, is more common than was previously thought (Mallet 2007), and is expected to become even more so in highly fragmented landscapes (Grant 1981; Arnold 1997).

Epigenetic processes are also triggered by the stresses of extreme climatic conditions (Grant-Downton and Dickinson 2006; Bossdorf, Richards et al. 2008; Chinnusamy and Zhu 2009; Richards, Bossdorf et al. 2010) (Fig. 1). In addition to the normal influences on phenological timing, temperature can, for example, indirectly impact on flowering time through the epigenetically-controlled heat shock response (Key, Lin et al. 1981; Chinnusamy and Zhu 2009).

Although much of our understanding of the contributions of epigenetic variation to the evolution of natural populations is still highly speculative (Richards, Bossdorf et al. 2010), the role of epigenetics clearly should not be ignored in an assessment of the evolutionary consequences of climate change.

### **Evolutionary impacts with reference to study species**

The foregoing investigations (Chapters 3 and 4) indicate that the flowering phenologies of *Diuris* and *Pyrus* provide multiple opportunities for, and sufficient instances of, enhanced genetic variability to allow expedient evolutionary adaptation. It is the extremes of temperatures and drought which are particularly potent selective factors, and both genera showed exceptional phenological responses under these adverse conditions. Endemic Australian natives such as *Diuris* have coevolved with, and are well adapted to, the extremes and unpredictability of the Australian climate; non-native species such as *Pyrus* are understandably more vulnerable.

Large and consistent temporal differences among locally adjacent, but distinct, orchid populations in the Belair National Park indicate local pools of genetic variation upon which natural selection can act (Thesis Chapter 3). The broader distribution of these resources through the process of hybridization will increase the number of individuals equipped to cope with the more extreme conditions expected along with future change (Blanchard and Runkle 2006; Hoffmann and Sgrò 2011). If, indeed, temporal synchrony for *Diuris* is enhanced under hotter and drier conditions as indicated in Chapter 3, the orchids seem well placed to acquire sufficient fitness through epigenetic-related processes to contend with future challenges. If, to the contrary, reproductive processes are negatively affected through disruptions of synchrony between sympatric species and their pollinators, this valuable genetic resource may be lost. Ensuing population decline may be delayed in the short term due to the longevity of individuals, their clonal capabilities, and their ability to self-fertilise to produce viable offspring. Less frequent crossing may also promote reproductive isolation, potentially driving the process of speciation (House 1997; Root, Price et al. 2003) and so further reducing successful hybridizing events.

Whatever the prospects, the long-term survival of *Diuris* and other native species, and the eco-services they provide, are highly dependent on the full gamut of evolutionary processes. Conversely, where phenology has economic consequences, horticultural practices and selection of suitable clones effectively annul their dependence on natural evolutionary processes. While introduced taxa such as *Pyrus* respond much more erratically to extremes of temperature and rainfall, with highly irregular flowering and fruiting events (Chapter 4), irrigation practices usually guard them from the vagaries of the weather. Other naturalized species which are similarly affected will undergo severe selective pressures (Opler, Frankie et al. 1976). However, in light of the multiple feedback loops, both positive and negative, any long-term prediction of the likely evolutionary impacts of climate change must be tentative indeed.



## **Chapter 6      Bioindicator species**

### **The canary in the coalmine**

*“There is ... growing appreciation ... for the role of phenology as a driver of ecological dynamics, as opposed to simply a response to environmental conditions. As such, phenology is a discipline that contributes fundamental insights into ecology at the individual level, but also scales up interactions to the population, community and landscape levels”* (Post and Inouye 2008).

One of the underlying assumptions and aims of my PhD research has been the selection of suitable bioindicators for monitoring the impacts of climate change on local South Australian species. However, insights arising from the foregoing components of this thesis indicate this intention may have been overly presumptive and premature.

### **Biological indicators overview**

The concept of using specific phenophases for bioindication long predated the current concerns of ecosystem integrity and global climate change. Throughout the millennia prior to meteorological recording devices and hourly weather forecasts, specific phenophases were considered useful as a means of estimating ‘the heat and the cold’, and the coming of the seasons: *“Now learn a parable of the Fig tree; When her branch is yet tender, and putteth forth leaves, ye know that summer is near”*. Mark 13: 28. Demarée and Rutishauser (2011) quote Morren’s reference to the work of Linnaeus (Linné 1788 p 328) who suggested *“producing in every country, every year, A Floral Calendar based on the precise moment of leafing, flowering, frutification and leaf fall, and, by observing at the same time, one would know the regions of the world. The climate will be well known by the botanist by the progress and the march of the year according to the time of*

*development of the leaves and their fall, from which one will estimate the heat and the cold.”* (Demarée and Rutishauser 2011).

Although highly sensitive technologies have now relegated this particular dependence on phenology to history, awakening concerns of global warming prompted de Groot et al. (1995) to present one of the first papers promoting the use of bioindicators to monitor the impacts of climate change. Their recommendations were made on the understanding that species which determine the structure and functioning of ecosystems could be used as bioindicators to assess the climate sensitivity of those systems.

## **Selection Criteria**

### ***Selection criteria; model candidates***

Ecologists have since established a broad set of criteria to guide the selection of good bioindicator species. Accordingly, phenological indicators should:

- a. provide a measurable response to specific climatic conditions which is easily observed and quantified.
- b. show a highly sensitive and unambiguous relationship between a readily observable phenophase such as flowering and a climatic variable, such as temperature <sup>1</sup> (Walkovsky 1998; Rumpff, Coates et al. 2008; Rumpff, Coates et al. 2010).
- c. show a sensitivity to changes in the climate, including temperature, precipitation and extreme events<sup>2</sup> which reflects the whole population response (Holt and Miller 2011).
- d. should be exclusively, or mainly, influenced by changes in climate, and less by biotic, edaphic and climate related events such as bushfires, ephemeral

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<sup>1</sup> This does not allow for possible interactions between temperature and rainfall

<sup>2</sup> Contradicts point b.

watercourses or floods (de Groot, Ketner et al. 1995; Rumpff, Coates et al. 2008; Rumpff, Coates et al. 2010).

- e. be insensitive to photoperiod (Osborne, Chuine et al. 2000).
- f. have an obvious, precise, synchronized flowering season of restricted time duration.

### ***Selection criteria: practical considerations***

To qualify as suitable candidates ‘in the field’, bioindicator species should also be:

- a. native or regionally well established, with wide and regular geographical distribution (de Groot, Ketner et al. 1995).
- b. abundant and common, with adequate and relatively stable local population density despite moderate climatic and environmental variability (Holt and Miller 2011).
- c. well-studied; their taxonomy well documented, and ecology and life history well understood (Holt and Miller 2011).
- d. well represented in archival records to provide a benchmark or reference point for future comparison (Gallagher, Hughes et al. 2009; Abbott and le Maitre 2010).
- e. easy to identify and observe for reliable, repeatable and cost effective monitoring. Species which may be confused with closely related species/cultivars/varieties should be avoided.

### ***Applications and assumptions***

Suppositions regarding the use of bioclimatic indicators for monitoring the long-term impacts of climate change abound in the literature. Recommendations often advocate the use of surrogacy for understanding responses in related species, and extrapolation from local to regional scales, or from one region to another. Statements such as “to track the impacts of changing climate on ecological processes”, or “for use as an early warning signal” are good overall summaries of intent, but are far too general to be of much practical use. While it may be highly desirable and expedient for indicator species to

satisfy these criteria, it is nevertheless a difficult task in practice (de Groot, Ketner et al. 1995), and futile without clear objectives (Holt and Miller 2011).

### **Bioclimatic indicator examples from the literature**

The following representative case studies explore various applications of flowering phenology for the purpose of bioindication. The first two, with specific goals for marketing and human health, are included to illustrate the broader relevance of bioindicators; the third to reveal the complexities involved when monitoring impacts on species fitness and the last to demonstrate the important insights to be obtained from historical records.

#### ***A Mediterranean species with implications for health***

Airborne pollen as a principal cause of allergies has a major impact on human health, and the timing of pollen release in Mediterranean regions has been closely monitored during recent decades (Osborne, Chuine et al. 2000). In this study they investigated the potential for olive flowering phenology to function as a sensitive indicator of future spring warming in the Mediterranean. They proposed and tested the use of sophisticated models to explore potential changes in phenology that could accompany future spring temperature changes in the region. Due to regional variation and possible local adaptation, they stressed the need to test a phenological model fitted to a single population of olive across a wide range of sites and cultivars for application at a regional scale; also the identification of geographical locations where phenology would be the most sensitive. They made the distinction between plasticity (fluctuations due to natural climate variability) and micro evolutionary process (the probable time scale of future trends in flowering date). The olive met the requirements of a good phenological indicator, suitable for use as a measure of the biological impacts of future climatic warming.

***Horticultural example***

Urhausen et al. (2011) investigated the relationship between climate variability and variations in phenological events in viticulture in the Upper Moselle region. Their primary aim was to predict the date of grape ripening and other phenological events dependent upon environmental conditions such as rainfall during the vegetative period. This was important on a number of counts, including the control of insect pests and fungal diseases.

***Multi species; three replicate studies***

Great tits (*Parus major*)

The following three separate studies replicate an investigation of the synchrony between *Parus major* fledglings and their food supply.

In the U.K, a 39 year study by Cresswell and McCleery (2008) showed the ability of great tits to maintain synchronization of hatch date with food supply by significantly varying incubation periods. The proportion of nests that had synchronized hatch dates depended on the yearly temperature pattern, and the principle nestling food supply, the winter moth (*Operophtera brumata*) caterpillars.

In Sweden, leafing phenology and timing of egg laying showed a strong relationship with spring temperature and the emergence of caterpillars (Nilsson and Källander 2006). The start of laying, and consequent reproductive success, could be predicted from leafing phenology. Swedish female tits could also strategically vary the clutch size, incubation period and hatch dates.

In the Netherlands, climate-related shifts disrupted the link between laying date and the peak in caterpillar food abundance (Visser, Holleman et al. 2006; Gienapp, Teplitsky et al. 2008). Here, the timing of the food peak and cue temperatures over a critical period for egg laying was found to have changed in the recent warm decades. The consequent

disruption of synchrony between offspring requirements and the caterpillar biomass had deleterious consequences, both in the number, and fitness, of fledglings.

***Historical data: information which may direct species choice and monitoring practice***

This important series of joint investigations has been based on a comparison of 19th century flowering records of Thoreau's Concord in Massachusetts with current phenologies (Nijhuis 2007; Miller-Rushing and Primack 2008; Willis, Ruhfel et al. 2008; Primack and Miller-Rushing 2012). These authors provide some interesting and practical insights concerning the manner in which a broad range of plant species are responding to global warming. Species with the ability to track short-term seasonal temperature variation have fared significantly better under recent warming trends (Willis, Ruhfel et al. 2008). On average, those most responsive to temperature in terms of their flowering phenology were found to have increased in abundance, or remained stable, over time, whereas species minimally affected by temperature, have declined in abundance or become locally extinct (Willis, Ruhfel et al. 2008; Primack and Miller-Rushing 2012).

The foregoing case-studies reveal some important insights. A good indicator species is ideally representative of a number of species within an ecosystem such that changes in the indicator species can infer changes in the other, non-measured species. These real-life examples serve to illustrate the impediments and challenges inherent in the practical applications of the theory. In relation to health, (e.g. airborne pollen and hay-fever management) and horticultural management practices (e.g. fruit ripening) objectives can be clearly stated, and outcomes are restricted to the bioindicators themselves. For environmental impacts, objectives are more intangible.

The replicated, broad-scale enquiries into *Parus* reproductive phenology illustrate a *major* difficulty arising from the complexity of ecological systems (de Groot, Ketner et al. 1995; Dale and Beyeler 2001). While leafing phenophase may be primarily temperature dependent, successful reproductive phenologies at the higher trophic levels of the moths and the tits have more complex dependencies. These case studies highlight the interactions

between hatch date, food supply and temperature which invalidate selection criteria b and c (page 158) and serve to caution that extrapolation from one region to another, even for the same set of species, is not in general a valid procedure.

Two further important issues derive from the Massachusetts studies. The positive correlation between temperature response and fitness over time provides an important focus for monitoring and research; both in the historical context and in the field. However, their well-supported claim that evolutionary relationships can *sometimes* (my emphasis) be used to predict species responses to future climate change (Willis, Ruhfel et al. 2008; Davis, Willis et al. 2010; Primack and Miller-Rushing 2012) should be employed with caution. Exceptions are found within the *Diuris* and *Pyrus* of my studies, and the foregoing case of *Parus major*.

### **Theory into Practice in Southern Australia**

Like canaries in a coal mine, bioindicators are expected to function as early-warning signals. Thus the effectiveness of an indicator depends on its ability to provide early signs that climate change is adversely disrupting the biological system (Visser 2008). According to Holt and Miller (2011), however, using a single species, or even a small group of species, to assess the changing impact of climate over time, represents “a gross oversimplification of a complex system”.

A great deal of work still needs to be done regarding the selection and application of bioindicators for monitoring. This is particularly so in Australia (Rumpff, Coates et al. 2008; Chambers and Keatley 2010; Rumpff, Coates et al. 2010) where synergistic responses to the combined effects of temperature and rainfall (Chapters 3,4) do not satisfy selection criteria a and b (see Selection criteria; model candidates p158).

More extensive, multi-species, case studies will be required in which appropriate field monitoring, herbaria archival records and statistical modelling will have large and

important roles to play. Investigations into the nesting phenology of *Parus major* and the flowering phenology at Massachusetts, are suggestive of the extensive spatial and temporal scales required for such endeavours. While the most appropriate indicators are deemed to have the most flexible responses to change; it is those with minimal sensitivity which are likely to be the most vulnerable (Rumpff, Coates et al. 2008; Rumpff, Coates et al. 2010; Primack and Miller-Rushing 2012).

In any long-term assessment, care must be taken when proceeding from specifics to generalizations. Species sensitivities to climatic variation are highly variable, and predictions as to how entire communities may respond to climate change will be difficult, even in relatively simple, well-studied systems (Bertness and Ewanchuk 2002). It is also necessary to determine to what extent a response of the indicator relates to the ecosystem. As bioindicator species invariably will have at least some differing habitat requirements from other species in the ecosystem (Holt and Miller 2011), the fitness of one does not automatically follow from the fitness of the other.

My research has consistently indicated that closely related species and, indeed, conspecific individuals growing in sympatry, are often differentially sensitive to precisely the same prevailing environmental parameters, suggesting that, for Australian conditions at least, extrapolations across taxa and regions should be employed with extreme caution, if at all.

### ***Potential bioindicator species for South Australia***

In an initial search for possible bioindicator species, I investigated flowering phenology of the exotics *Arbutus unedo* and *Erythrina variegata*, and natives *Angophora floribunda*, *Brachychiton discolor* and *Capparis mitchellii*. The data sets were obtained from the archives of the University of Adelaide Waite Arboretum. Due to the extended periods over which flowering was recorded (full flowering varied from five months for *B. discolor* to ten months for *A. floribunda*) and the limited number of individual trees (nine in total), phenological responses to temperature and rainfall were inconclusive. There was, however, some evidence that flowering intensity of *B. discolor* and *E. variegata* was



impacted by the El Niño/La Niña cycle. As intensity is not a phenophase, it was not investigated further, but this aspect of flowering should perhaps be taken into consideration when reliable data is available.

The ClimateWatch website <http://www.climatewatch.org.au/species/plants> provides an extensive list of phenological indicators which invites broad-scale monitoring by Australian citizens. This important collection and storage system has the added benefit of raising public awareness and providing a sense of public ownership. I do not, however, consider the majority of listed plant species to be suitable as environmental indicators for monitoring South Australian ecosystems, and have suggested some appropriate alternatives in Table 1. While many of the ornamental species, such as the Illawarra Flame Tree, Jacaranda and White Cedar, can be found growing as street trees or in parks and gardens where they can be readily observed, they often receive supplementary watering during dry periods. Thus, their phenology does not reflect a response to natural environmental conditions.

In Table 1 I have suggested related substitute species for South Australia, based on their broad distribution, abundance, and ease of recognition and monitoring. This list is by no means exhaustive, and is included here as a guide only. Such endeavours provide an opportunity to determine the extent to which related species in different regions respond to climate change, and hence their suitability as reliable bioindicators. Many of the suggested species have long flowering seasons, limiting their usefulness and requiring first-flowering, as opposed to peak flowering, observations. Wild gladiolus, and invasive *Senecio* species have been included, as detailed knowledge of their phenology may contribute to our understanding of their success, and assist with their eradication or control.

**Table 1** South Australian endemic species representative of, or substituted for, some of the plants listed by ClimateWatch <http://www.climatewatch.org.au/species/plants>.

Listed by ClimateWatch	Suggested South Australian species
<i>Banksia</i>	<i>Banksia. marginata</i> , <i>B. ornata</i>
Bearded Heath, Fushia Heath	<i>Epacris impressa</i>
Christmas Bush	<i>Bursaria spinosa</i>
Coastal Tea Tree	<i>Leptospermum coriaceum</i> , <i>L. lanigerum</i> ; <i>Melaleuca lanceolata</i> , <i>M. wilsonii</i>
Emubush	<i>Eremophila glabra</i> , <i>E. longifolia</i>
Native Buttercup	<i>Hibbertia sericea</i> var. <i>sericea</i>
Native Wistaria	<i>Hardenbergia violacea</i>
Pea plants	<i>Eutaxia microphylla</i> ; <i>Kennedia prostrata</i> ; <i>Platylobium obtusangulum</i> ; <i>Pultenaea daphnoides</i> , <i>P. largiflorens</i>
Sturt's Desert Pea	<i>Swainsona formosa</i>
Wild Gladiolus	also invasive <i>Senecio</i> species
Additional species	<i>Clematis microphylla</i> ; <i>Lasiopetalum baureri</i> , <i>L. behrii</i> ; <i>Tetralthea pilosa</i> ,

### *The special case of Diuris orchids*

Because of the popularity of Australian native orchids among collectors, field naturalists, and the general public, their potential role as bioindicator species is worthy of consideration. A large number of species are already on the endangered list <http://www.flora.sa.gov.au/census.shtml> (accessed 20 September 2012), and conservation measures are of high priority (Quarmby 2010). Five of the 10 South Australian *Diuris* species are currently listed: three as endangered, one as rare, and *D. behrii* as vulnerable. The question therefore arises: can a bioindicator be selected from a responsive and

abundant representative of the genus which is capable of signalling the further decline of the vulnerable *D. behrii*?

As a contender, *Diuris orientis* ticks many of the boxes required of indicators. However, non-linear responses to both temperature and rainfall thresholds for earliest flowering, and geographical influences (Chapter 3), serve to negate the positives, and challenge predictive ability and hence its usefulness as an indicator. The role these food deceptive, non-rewarding, orchids play in the ecological network presents a further challenge. Specialist requirements restrict their dependence upon a relatively small number of pollinators which do not receive a reward for services rendered: the orchid's gain is the pollinator's loss. This relationship accentuates the difficulty of translating the fitness level of the orchid to that of other species in the network. This difficulty is inflated at large spatial scales, as *Diuris* species were found to have greater ongoing fitness in southern regions (Chapter 3). The three *Diuris* study species also showed different levels, and sometimes different directions of response, invalidating surrogacy of one species for another. Gallagher et al. (2009) likewise anticipated that Orchidaceae may provide useful indicator species, but failed to identify any among Australian alpine species.

Orchid populations in Belair National Park show a similar directional correlation between phenological plasticity and abundance as do plant communities in Massachusetts, i.e. the most abundant species, *D. orientis* is also the most responsive to temperature, while *D. behrii* was the least responsive, the least abundant and the most vulnerable. This is a matter of immediate concern, not ameliorated by the inadequacy of *D. orientis* as a potential bioindicator. Measures of phenological plasticity may be used to identify potentially vulnerable populations or species; those species least unable to track the changing climate considered to be under the greatest potential risk.

In summary, I present some guidelines applicable to phenological monitoring of ecosystem functioning in southern regions of Australia. The list is not inclusive, and is not intended to trivialize the challenges to be encountered in the selection of appropriate indicators on the scale of the ecosystem.

***Stage 1: Identification of potentially suitable bioclimatic phenological indicators***

With reference to both herbarium data sets and other relevant observations:

- a. Select a range of candidates (multiple species) as potential indicators for the region under consideration.
- b. Check for exceptional phenological response to droughts and extreme summer heat, and consider how this may compromise indicator effectiveness.
- c. Develop and explore appropriate predictive models specifically tailored for the situation and potential candidates (Osborne, Chuine et al. 2000). Both temperature and rainfall parameters, main and interactive, must be included in the models.
- d. Establish realistic benchmarks or reference points for ongoing monitoring (Osborne, Chuine et al. 2000; Gallagher, Hughes et al. 2009; Abbott and le Maitre 2010). At a minimum this should include median and range of flowering dates plus measures of abundance.

***Stage 2: field monitoring***

- a. Address the precise reasons for the monitoring (Abbott and le Maitre 2010; Holt and Miller 2011).
- b. Decide in advance which outcomes should trigger alarm bells, such as rare species unable to track short-term seasonal temperature variation (Møller, Rubolini et al. 2008; Willis, Ruhfel et al. 2008) and casualties to extreme events at the range limits.
- c. Monitor groups of interacting species rather than individual species.
- d. Consider keystone species, networks, generalists vs. specialists, and different trophic levels to gauge the impact of climate change on biodiversity and ecosystem processes (Abbott and le Maitre 2010).
- e. Monitor the leading and trailing edges of a species range where extreme, environmental factors such as frost, heat, drought, floods etc. are of greatest consequence, and where climate-related changes can be first detected. This also enables space for time interpretations (de Groot, Ketner et al. 1995).

- f. Identify other geographical locations where phenology could be highly sensitive (Osborne, Chuine et al. 2000).
- g. Identify and prioritize subpopulations and individuals with widely differing phenological responses to the prevailing climate.

### ***Stage 3: interpretation and feedback***

- a. Must be able to interpret the responses of indicators with regard to the fitness of the population and/or species (Holt and Miller 2011). Clarify ‘alarm bell’ responses which signal deleterious ecological consequences.
- b. Gauge the current and ongoing fitness of the populations and/or species with respect to the established benchmark or reference point.
- c. Determine the appropriateness or otherwise of surrogacy for related species, and potential application of the indicator across different spatial scales and regions.
- d. Adjust the monitoring program as knowledge accumulates.



## Concluding remarks

When beginning my PhD, the overall concern of phenological research was the occurrence, magnitude and direction of shifts in relation to global warming. It has now been well established that the ability to regulate the timing of reproductive phenophases is a vital part of a plants defences to survive the vagaries of climate, and research methods have shifted along with the phenology. More highly sophisticated statistical techniques are being employed to gauge the intricacies of phenological responses to highly specific climatic triggers. With the trial of GAMLSS statistical modelling, our work has pioneered the applicability of some of these techniques to large, non-linear data sets, and in doing so, has contributed to a better understanding of phenological responses in southern Australia, particularly under conditions of frequent, and at times severe, water-limitations.

In general, however, phenological research still remains focussed on individual species, and has not yet progressed to the more practical application of this knowledge to ecosystem monitoring. The true measure of the value of phenological research will lie in its application to the ecosystem and conservation management practices. Despite having made considerable progress towards the goal expressed by Clarke in 1893 (page 1), it still remains for the researches of botany to show decisively how the association between flowering plants and the seasons of the year “*accords with the eternal fitness of things.*” (Clarke 1893) p769.

In fact, most of today’s researchers would be satisfied to discern how phenological responses may ‘accord with fitness’ within the time frames of current scenarios outlined by the Intergovernmental Panel on Climate Change (IPCC). The present imbalance of examples from our region of the world may give a false impression to the leaders, citizens and students of this country that the ecological impacts of climate change are remote, thus diminishing its relevance and urgency. With access to modern computer programs such as

the BRAHMS 7 DOCUMENTATION phenological predictability index for herbarium-based phenological studies BRAHMS (2012), researchers should be better equipped to undertake the necessary broad-scale, ecological explorations (Proença, Filer et al. 2012) necessary to develop strategies for field monitoring and conservation practices. The contribution of the nation's herbaria to this endeavour will continue to be of the highest importance.

A local South Australian long-term research initiative, – Transects for Environmental Monitoring and Decision Making (TREND) <http://www.trendsa.org.au/> – has been established as a component of the Australia wide Terrestrial Ecosystems Research Network (TERN) <http://tern.org.au/>. TREND transects have been sited to incorporate a variety of specific environmental gradients, ranging from the higher rainfall regions of the southern Mount Lofty Ranges to the hotter and drier northern Flinders Ranges. Methodologies to monitor changes over time along these transects include site photo panoramas ideally situated to record ongoing phenological changes, and potential impacts thereof.

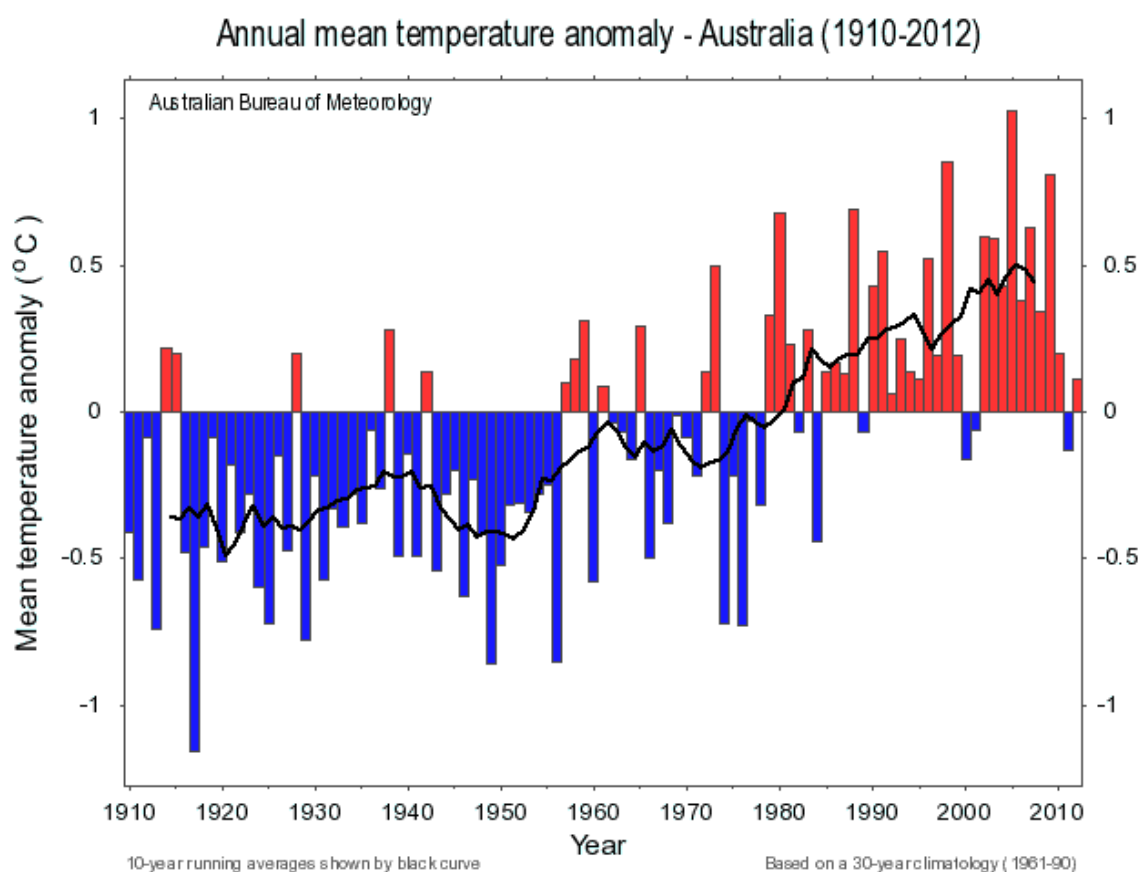


## Appendices

### Appendix A

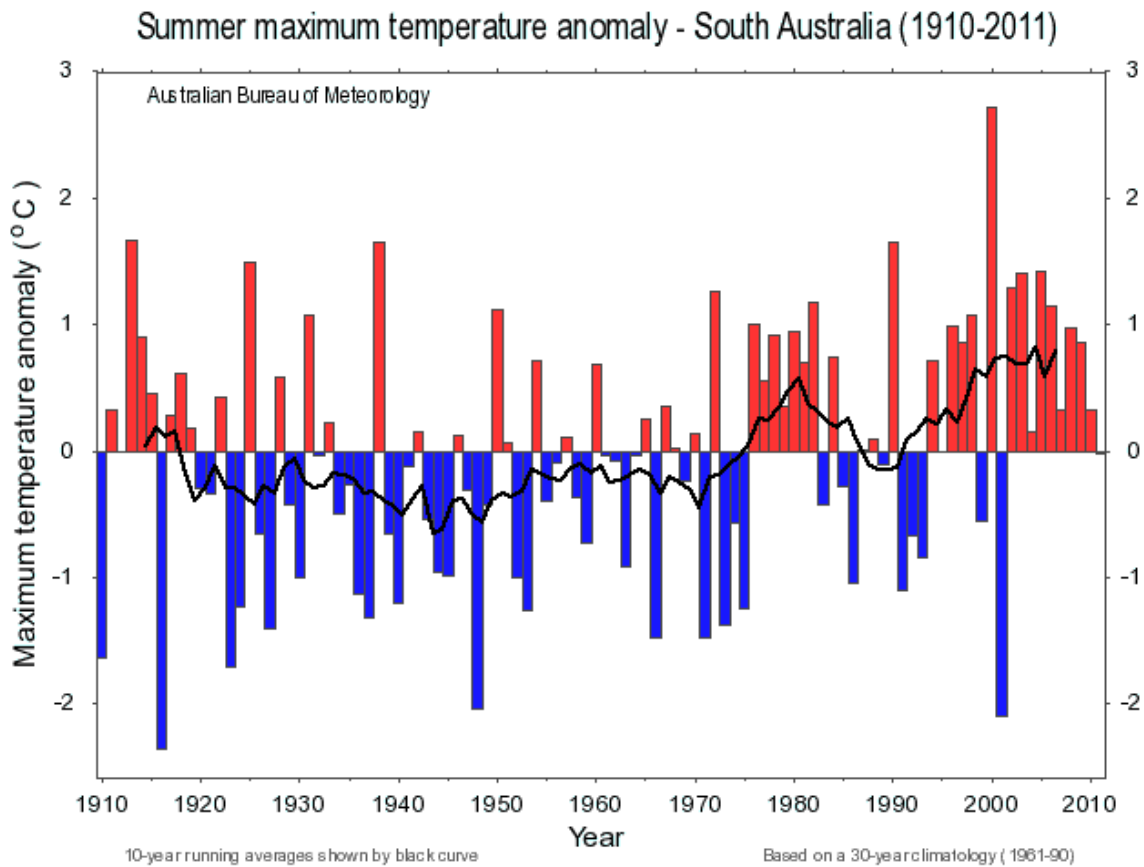
The following plots were obtained from the Bureau of Meteorology  
[http://www.bom.gov.au/climate/averages/tables/cw\\_023090.shtml](http://www.bom.gov.au/climate/averages/tables/cw_023090.shtml)

#### A.1 Annual mean temperature anomaly – Australia



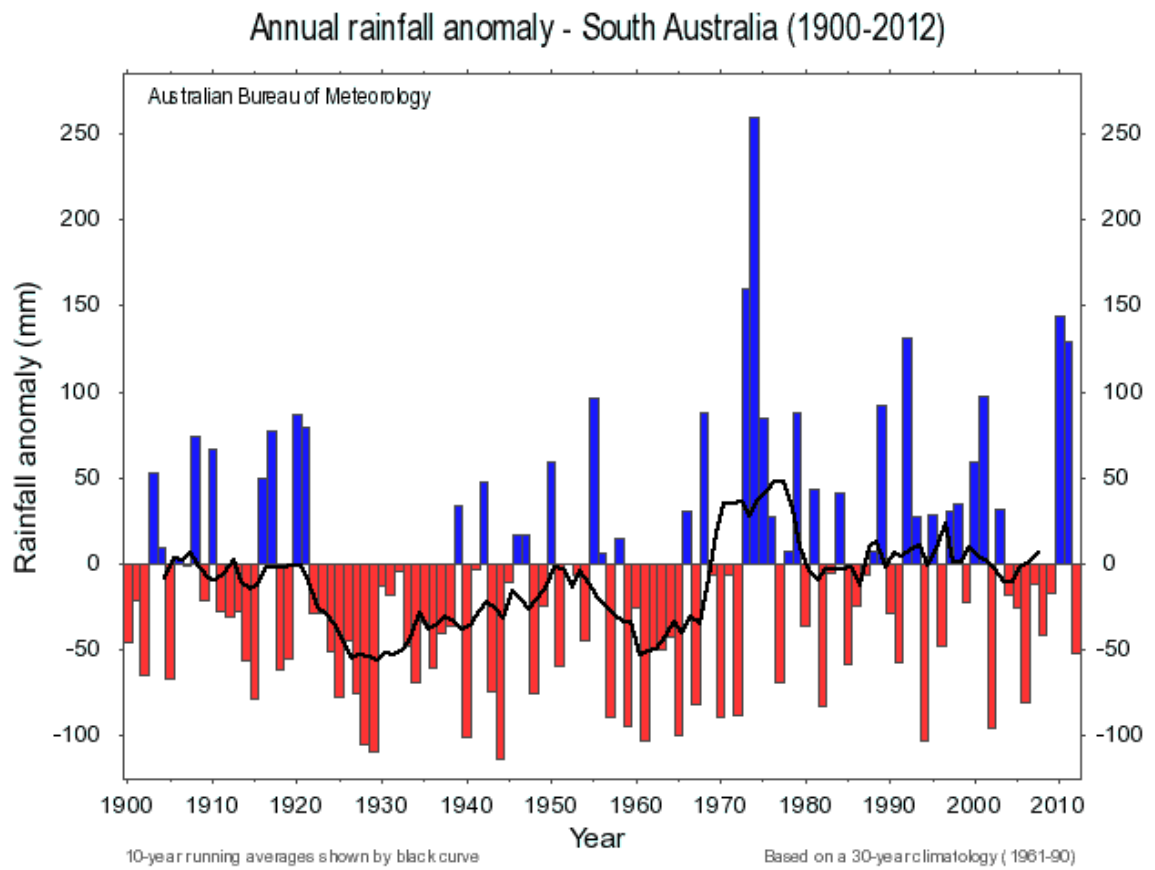
**Fig. A.1** Australian annual mean temperature anomaly (1910-2012) with 10-year running average based on a 30-year climatology (1961-1990). Average (1961-90): 21.8 °C.

**A.2 Summer maximum temperature anomaly – South Australia**



**Fig. A.2** Maximum summer temperature anomalies in South Australia (1910-2011) with 10-running average, based on a 30-year climatology (1961-1990). Average (1961-90): 34.0 °C.

### A.3 Annual rainfall anomaly – South Australia



**Fig. A.3** Rainfall anomalies for South Australia (1900-2012) with 10-year running average, based on a 30-year climatology (1961-1990). Average (1961-90): 225.2 mm.

#### *A.4 Bureau of Meteorology temperature and rainfall statistics for Adelaide*

Temperature and rainfall records spanning the period covered by the phenological data were not available from any one station. Temperature records were therefore obtained from the Adelaide (Kent Town) station # 23090 and rainfall records from the station at Glen Osmond # 23005 station situated approximately 5km south of Kent Town.

The station at Glen Osmond, situated approximately 1 km from the Waite Arboretum, is the nearest station to the *Pyrus* plantation.

[http://www.bom.gov.au/climate/averages/tables/cw\\_023090.shtml](http://www.bom.gov.au/climate/averages/tables/cw_023090.shtml)

##### *Temperature*

Adelaide (Kent Town): (23090) temperature

Mean maximum temperature 22.3<sup>0</sup>C

Mean minimum Temperature 12.2<sup>0</sup>C

Highest maximum temperature: 45.7<sup>0</sup>C 28 Jan 2009

Lowest minimum temperature: -0.4<sup>0</sup>C 08 June 1982

##### *Rainfall*

Glen Osmond (23005)

Mean annual rainfall: 628.3mm

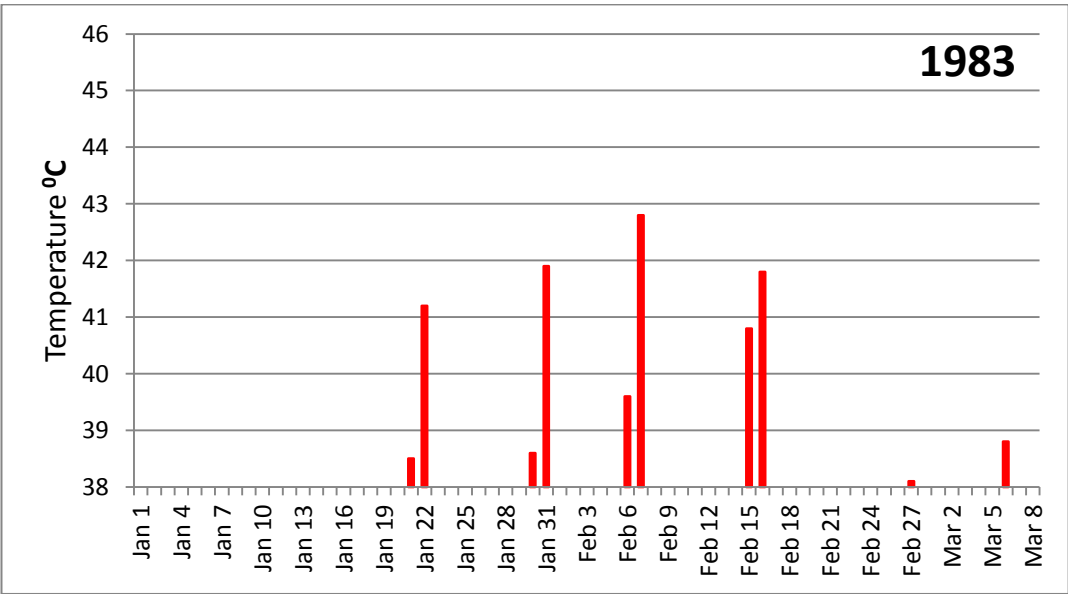
Lowest: 304.0mm

Highest 1010.1mm

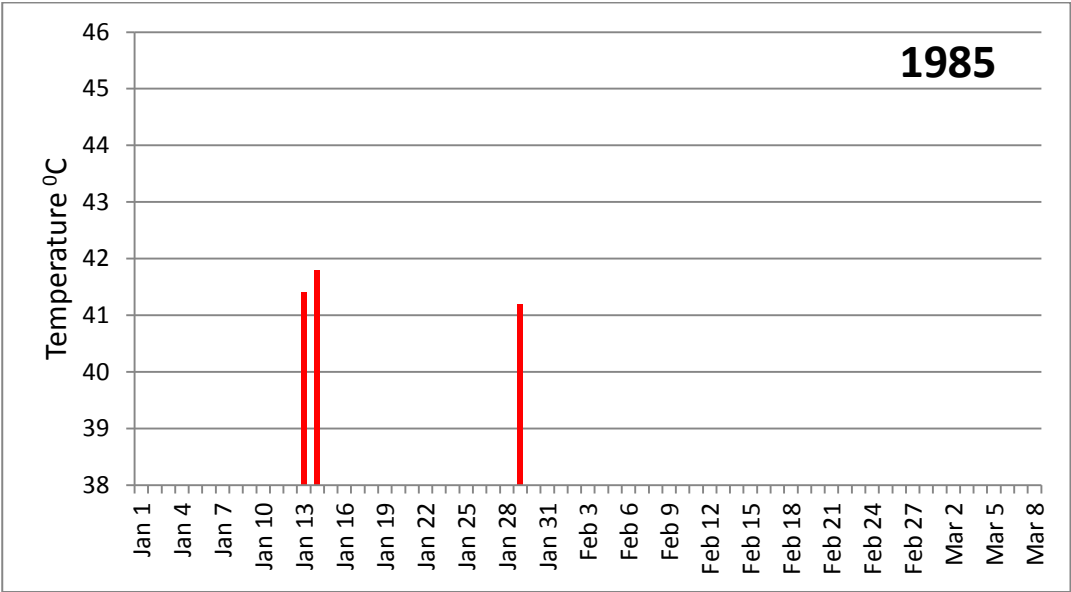
Appendix B

Summer temperature plots

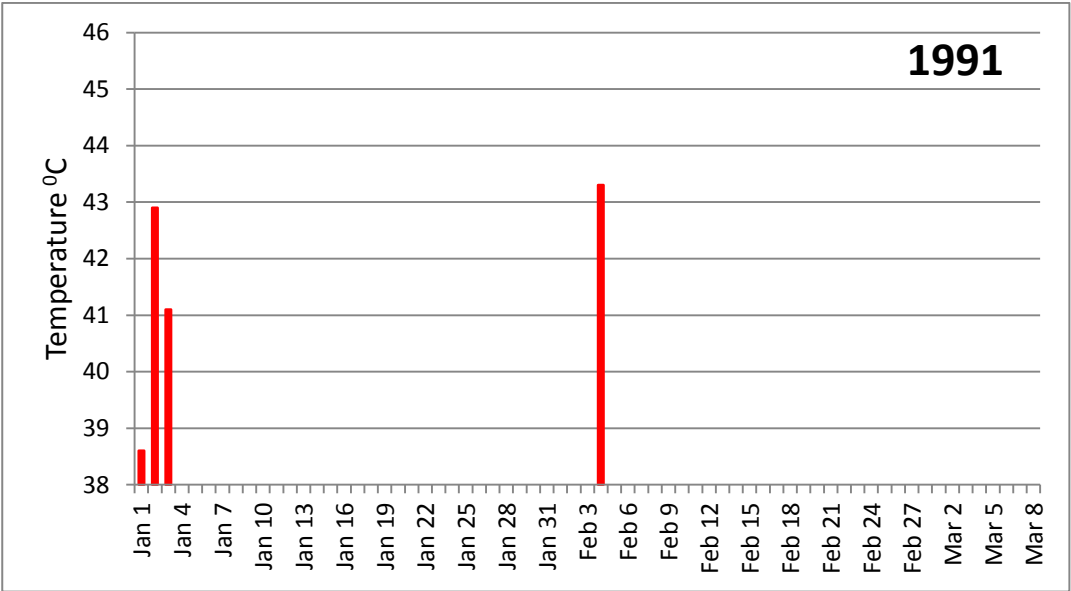
Plots showing maximum temperatures above 37°C (recorded at Adelaide, Kent Town) for the years in which summer/autumn flowering was recorded for *Pyrus* trees growing at the Waite Arboretum.



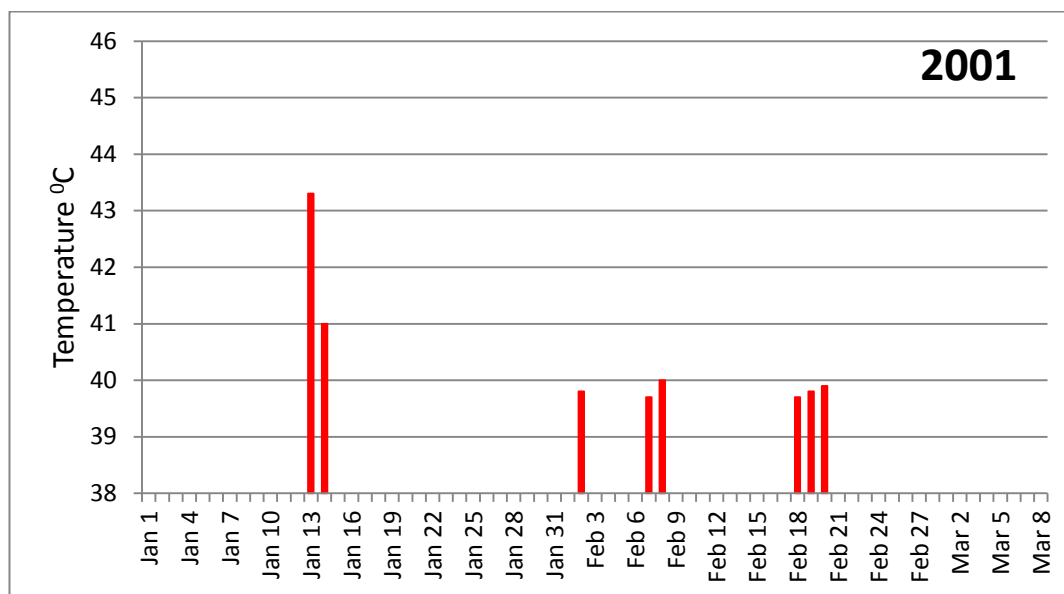
Full Flowering observed 18, 20, 31 March, 12 April, 1983



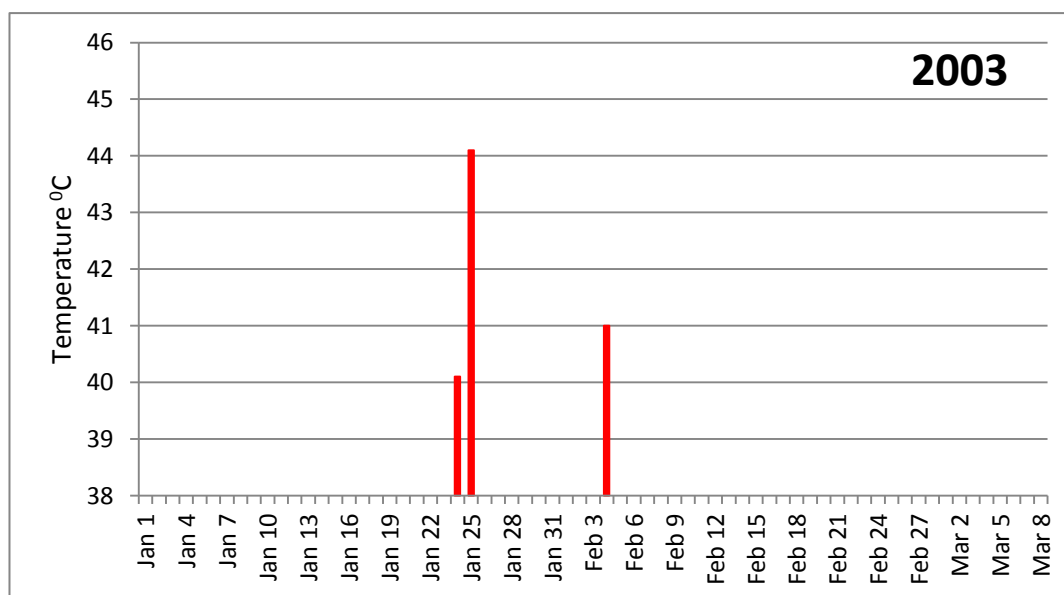
Full Flowering recorded 12 April 1985



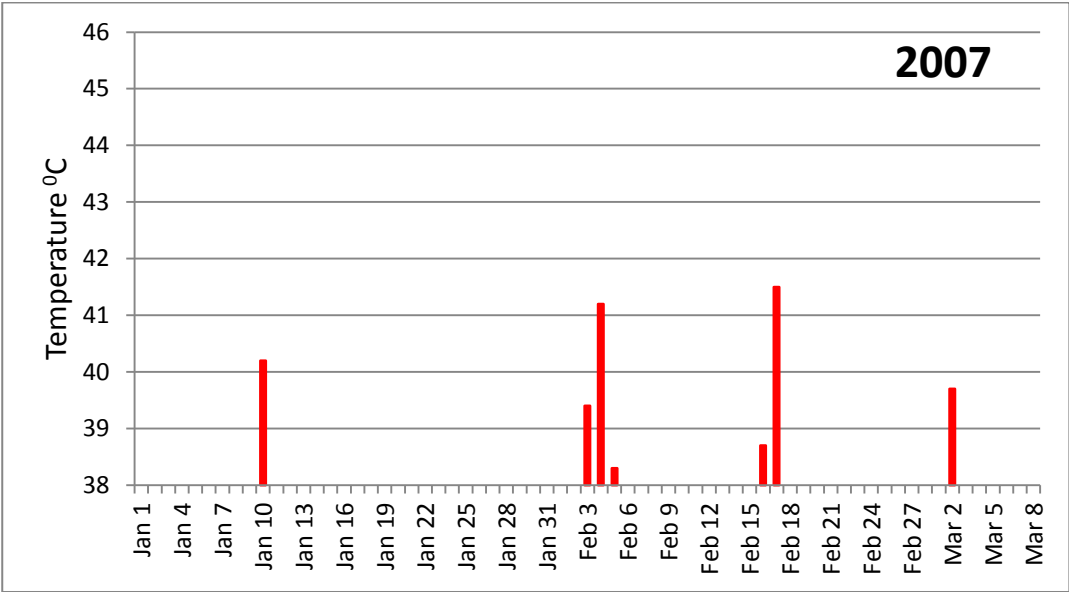
Full Flowering recorded 18 April, 1991



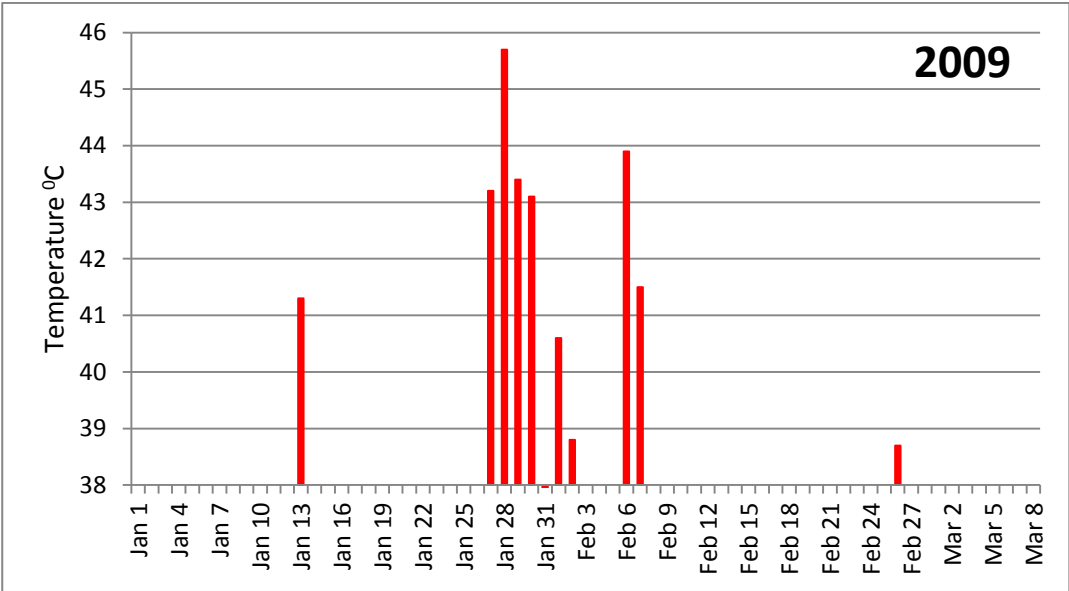
Heavy flowering ending recorded 27 April, 2001



Full Flowering recorded 27 March, 2003



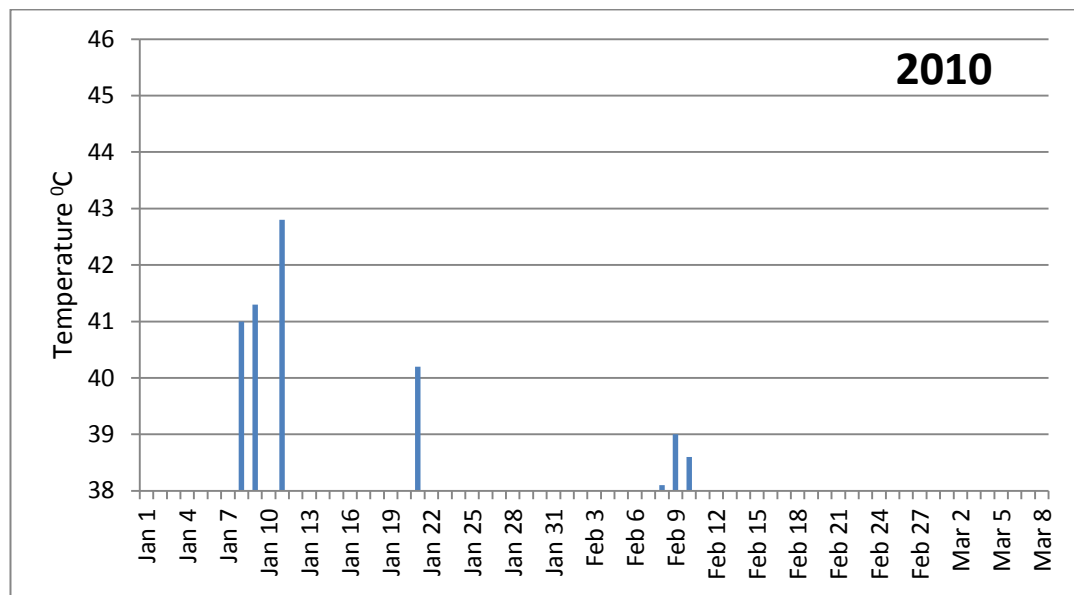
Heavy flowering beginning recorded 19 March, 2007



Flowering beginning 18 Feb; Full flowering/flowering 1, 6, March, 2009



## Appendices



No summer flowering observed in 2010. This year was the only recorded year in which trees were observed and found not to be flowering during the summer months.



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