

## Temperature and Orchids 1: Winter Chilling, Global Warming and Hardy Orchids (especially *Cypripedium calceolus*) David Trudgill

Many British orchid species become dormant in the autumn and this article firstly explores the role of changing day-length and winter chilling in breaking dormancy/initiating bud growth in the spring. The mini experiments described here involve mature plants lifted from the paths in our meadow and grown in pots. The second part of this article examines the implications of our warming climate for orchid species such as *Cypripedium calceolus* that require a period of chilling before they can re-start growth in the spring.

### Winter Chilling

Eight pots growing *Dactylorhiza* spp. (a mixture of *D. purpurella* and *D. fuchsii*) were split into two groups of four pots. One group was maintained from October 2016 in an unheated out-building, the other on a cool, north facing window sill within our house (temperature >9°C). All plants were moved to a cold-frame in early April 2017. The experiment was repeated in 2017/8 (but with only three replicates), and also included three replicates of *Platanthera bifolia*. The results were similar for both years. The buds of the *Dactylorhiza* spp. kept in the out-building started to appear above the soil surface in late-March as temperatures increased; the *P. bifolia* two or three weeks later. In contrast, the buds of the plants in our house appeared above the soil surface several weeks earlier. However, as is shown in Figures 1 and 2 (the second experiment photographed on 20<sup>th</sup> May 2018) the subsequent growth of the indoor plants was much slower and they were stunted compared with those kept in the out-building.

These results support observations by Rasmussen (1995) that during micro-propagation of hardy orchids many species, including *Cypripedium calceolus* (Lady's-slipper Orchid) and species of *Dactylorhiza*, required a period of chilling before growth will re-start in the spring. Typically, the tubers or rhizomes with buds need two to three months in a fridge at <5°C. John Haggard in his micro-propagation protocol states 'the protocorms of summer-green species will not produce roots and leaves until they have a winter simulating cold period – usually three months in the refrigerator'. However, in my experiments the plants that were kept in-doors through the winter did not remain fully dormant, indicating that the passage of time may also be a factor initiating growth.

Fig. 1: *Dactylorhiza* spp.      Fig. 2: *Platanthera bifolia*

In both cases the three pots at the back were maintained in an unheated out-building from mid-October to mid-April and the three in the front were kept in our house.



### Day-length

The potential influence of day-length was also tested on *Dactylorhiza* spp. (a mixture of *D. purpurella* and *D. fuchsii*) in pots plunged in soil out of doors. In mid-October 2016 light-proof covers were placed over three pots and three were left uncovered. In the spring of 2017 there was no apparent difference in the timing of bud emergence from the soil but, when the plants were photographed on the 17<sup>th</sup> April 2017 it was evident that the shoots of those held in the dark had become elongated (Figure 3). Therefore, increasing day-length appears not to be a factor initiating bud growth in the spring.



Figure 3. The three pots of *Dactylorhiza* spp. on the right were kept in the dark from mid-October to early April, and the three on the on left were kept in the light. Thereafter they were all in the light until 17th April. The meadow from which the plants came is in the background with Cowslips coming into flower.

### Increasing Winter Temperatures

The average annual temperature of the UK has increased ca. 1.5°C since the 1880s creating opportunities for orchids to extend their distributions northwards (Trudgill 2017). However, for orchids with a chilling requirement it is the minimum winter temperatures that are crucial. The historic records available for each of the UK's

met. stations provide two average temperature values for each month – the averages of: 1) the daily minimum and 2) the daily maximum. In Table 1 (A) I present the average minimum temperatures and (B) the average mean temperature (mean of the minimum and maximum) for the three coldest months for the periods 1899-1918 and 1999-2018 from five met. stations. These met. stations were selected because they had all started before 1890 (see – <https://www.metoffice.gov.uk/research/climate/maps-and-data>)

	Altitude	A) Average lowest temperature		B) Average mean temperature *	
		1899-1918	1999-2018	1899-1918	1999-2018
Armagh	62m	1.4	1.9	4.3	5.1
Durham	102m	0.3	1.2	3.2	4.2
Oxford	63m	1.5	2.2	4.3	5.3
Sheffield	131m	1.4	2.2	4.0	4.8
Stornoway	15m	1.4	2.4	4.3	5.0
Mean		1.2	2.0	4.0	4.9
<b>C)</b>					
Eastbourne	7m		3.8		6.3
Valley	10m		3.7		6.1

Table 1: A) Average lowest, and B) average\*mean temperature (°C) for the three coldest winter months for two 20-year periods 100 years apart at five sites, and C) for Eastbourne and Valley for 1999-2018.

\*The met. office provides a mean of the warmest and the coldest temperatures for each month. The monthly mean is calculated as the average of the two. Here I give only the averages of the three coldest winter months.

Of these five met. stations Durham had the coldest winter weather (Table 1), reflecting its altitude and location in the east of Britain. The overall average for these five sites indicates that the average minimum temperature for the three coldest months had increased by 0.8°C and the average mean temperature had increased by 0.9°C. At two sites (Armagh and Oxford) the mean temperature for the coldest three months in the period 1999 to 2018 was now >5°C (Table1 ).

### Days with an Air Frost

The met. station historic data also gives the number of days per year with an air frost. Stornoway had the fewest and Durham the most. The average number of frosty days for the five sites in the period 1990 to 2016 (33.8 days) had decreased by 24% compared with the period 1890 to 1929 (44.5 days).

### **Met. Stations in Areas with Warmer Winter Weather**

The five met. stations for which long-term data is available were in very different parts of the UK, and at different altitudes, but none were in the mildest parts of Britain i.e. the south coast of England and the west coast of Wales. Here I present data for two met. stations – Eastbourne (50.76N, 7m above sea level on the south coast of England) and Valley (53.25N, 10m above sea level on the Anglesey coast, Wales) – that have relatively mild winters. The average minimum and mean temperatures for the period 1999 to 2018 were 3.8°C and 6.3°C for Eastbourne, and 3.7°C and 6.1°C for Valley (Table 1 (C)), and there was an average of only 14 and 18 days with an air frost respectively. There were year to year differences and the average minimum temperature for any of the three coldest months never fell below 5°C at Valley in 2007 (mean 5.0°C) and in 2014 (mean 5.0°C), and in Eastbourne in 1990 (mean 5.9°C) and 2007 (mean 5.4°C).

### ***Cypripedium calceolus***

The data for all seven met stations shows that UK winters have become less cold. Consequently, winter temperatures on the coastal areas represented by the Eastbourne and Valley met. stations may, in years with mild winters, be too high to provide adequate chilling for orchid species with this requirement. The station at Valley in Anglesey is the most representative for Gait Barrows (54.20N, alt. 54m) that is one of the areas for the re-introduction of *C. calceolus*. Although Gait Barrows is c. 100km further north and 50m higher than the met station at Valley, it still seems likely that the mean temperature for the three coldest months at Gait Barrows might now be >5.0°C, close to the upper threshold for adequate chilling. Harrap & Harrap (2009) comment that *C. calceolus* ‘is absent from the Atlantic fringes of Europe and that the English populations were therefore always out on a limb’. Following an exceptionally mild winter in southern Sweden in 2007-8 nearly 400 plants of *C. tibeticum* and *C. macranthos* ‘types’ did not emerge in the spring and died due to inadequate chilling (Malmgren & Nystrom, see [http://www.lideforestgarden.com/orchis/cypripedium\\_eng.htm](http://www.lideforestgarden.com/orchis/cypripedium_eng.htm)). For the last ten years Irmin Vogler, who has long experience of growing *Cypripedium* spp. in the Berlin area of Germany, has no longer been able to successfully grow, out-doors, plants of several *Cypripedium* spp. and hybrids, including *C. calceolus*, because the shoots have become much weaker (pers. comm.). The Berlin winters have become wetter and milder and do not seem to be cold enough to provide ‘a successful over-winter period’ (I. Vogler pers. comm.).

### **Winter Chilling Discussion**

The simple experiment I did supports the suggestion that orchid species that become dormant in the winter require a period of chilling to help overcome that dormancy and ensure normal growth. However, although stunted when not chilled, they did grow and this may have been because plants have an ‘internal (circadian) clock’ and are able to sense the passage of time (McClung 2006).

If we focus on *C. calceolus*, the data from the Durham met. station clearly indicates that UK winters in north east England are still cold enough for it to receive adequate chilling. The west of England is much milder and the temperature data for the met. station at Valley on Anglesey suggests that chilling might be inadequate in some winters. However, Ian Taylor (Natural England) commented that the plants that had been re-introduced at Gait Barrows (NW Lancashire) had not (yet) shown any problems attributable to insufficient chilling. There are several uncertainties when trying to interpret the biological impact of data from met. stations e.g. they measure air temperatures at 2m above the ground, and temperatures at the soil surface may, especially on nights with clear skies, be less than those recorded. Also, as observed with *Arabidopsis thaliana* (Wollenberg & Amasino 2012), temperatures >5°C might still provide adequate chilling, only requiring longer exposure, and populations from different climatic zones may have been selected for appropriate but different chilling requirements (Malmgren pers. comm., Stinchcombe *et al.* 2005).

### **Wider Considerations**

Global warming has resulted in a northerly shift in the distribution of many butterflies in the northern hemisphere. Of greater relevance to this article is the observation that the southern edge of their distributions has also shifted northwards (Franco *et al.* 2006; Parmesan *et al.* 1999). This may be due to increased summer temperatures being less favourable for some species with the result that they are out-competed by species adapted to warmer conditions.

In contrast, despite temperatures increasing, the distribution boundaries of many orchids in Britain and Western Europe have not moved northwards, and some have declined. Changes in land-use are probably responsible (Bell 2015; Vogt-Schilb *et al.* 2015). Vogt-Schilb *et al.* found that the declines were greatest for rare, protected species, despite their status. Currently, the guidelines for re-establishing species require them to be re-introduced into areas within their historic range (see Maschinski & Albrecht 2017). Our changing climate will, I suggest, require a more forward looking and rational approach to conservation and management, including translocating species to more northerly areas outside their historic range. From an analysis of the BSBI distribution records Bell (2015) observed that some plant species had northerly out-posts that were attributed to ‘assisted migration’. It is possible that spread of *Ophrys apifera* (Bee Orchid) into eastern Scotland is one such instance as the new sites are all adjacent to the A1 and/or to power stations (Trudgill 2017). However it got there, it appears to have received a warm welcome (Anon. 2017)!

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## **False Pugsley’s Marsh-orchid** **Richard Bateman**

My recent article in *JHOS* on *Dactylorhiza* systematics (Bateman 2019) focused primarily on explaining how recent advances in next-generation DNA sequencing had clarified our understanding of the evolution of the genus in western Europe (Brandrud *et al.* 2019). Consequently, I dipped into specific taxonomic considerations only when attempting to refute recent attempts to resurrect the Hebridean Marsh-orchid – a greatly over-inflated taxon that, in 2006, I so foolishly suggested could merit the exalted status of full species despite its extreme rarity. However, this is not the issue that has sparked such a vibrant and intriguing debate on the HOS Discussion Forum this month (June 2020). That debate has instead focused on other aspects of the narrow-leaved marsh-orchids – more specifically, questioning the most appropriate taxonomic status for *D. praetermissa* subsp. *schoenophila* (which might usefully be named False Pugsley’s Marsh-orchid!) and how best to distinguish it from genuine Pugsley’s Marsh-orchid, *D. traunsteinerioides*. Given my parental responsibility for having circumscribed and named *schoenophila* (Bateman & Denholm 2012), I think it only right that I now attempt to explain the observations and thoughts that caused it to be singled out from the recalcitrant crowd of infuriatingly subtle morphological variants that together constitute that most Gordian of Knots, the tetraploid marsh-orchids.

### **Background**

The active part of the story began in June 2005, when I guided Mikael Hedrén and Sofie Nordström – two colleagues from the University of Lund, Sweden – on a madcap, week-long tour that encompassed most of England. We were in search of tetraploid marsh-orchids to study and sample; my colleagues collected DNA material while I feverishly accumulated data from the same plants using the 52 morphometric characters established for the genus two decades earlier by Bateman & Denholm (1983). The pace of the tour meant that I was rarely able to meet my usual target of measuring ten plants per population; only on the final afternoon did the pace slacken, allowing my Swedish colleagues to visit the Eagle – the Cambridge pub where, in 1953, Francis Crick famously announced to a somewhat underwhelmed clientele that he and James Watson had discovered “the secret of life” (more precisely, the molecular structure and function of DNA).

Although the aim of our tour was to obtain a good representative sample of English dactylorchids, we especially targeted populations that had, with varying degrees of confidence, been attributed to Pugsley’s Marsh-orchid, *D. traunsteinerioides*. We awarded this species star billing because it appeared to cause so many ongoing difficulties of identification and thus of botanical mapping. Distribution maps of the day showed an improbably sporadic distribution of the species across the whole





of the British Isles, with concentrations mapped in parts of Ireland, North Wales, East Anglia, North Yorkshire and, more recently, the western seaboard of Scotland. Almost every aspect of these populations had been repeatedly questioned throughout the 20<sup>th</sup> century so it seemed a good idea to celebrate the beginning of the 21<sup>st</sup> century by attempting to solve at least some parts of this long-running puzzle.

Integrating these data with further populations from throughout the British Isles meant that our work was not published for a further six years (Hedrén, Nordström & Bateman 2011), and even then, the DNA work was unaccompanied by the parallel morphometric work. Anyone reading the paper should be aware that the senior author of the research paper was Scandinavian so, to the detriment of my blood pressure, names that predominate in Scandinavia also predominated in the paper; thus, *D. fuchsii* became *D. maculata* subsp. *fuchsii*, and *praetermissa*, *traunsteinerioides*, *purpurella* and *kerryensis* (still operating under its former name of *occidentalis*) were treated as subspecies of *D. majalis*. Sadly, similar taxonomic decisions were also taken more recently by Kühn *et al.* (2019), who were then obliged by the rules of nomenclature (not to be confused with genuine science) to not only return *D. kerryensis* to *D. majalis* subsp. *occidentalis* but also to rename *D. praetermissa* as *D. majalis* subsp. *integrata* (don't you just love nomenclature?!). Also, the much-discussed "*ebudensis*" was still at that time treated by us as a fifth subspecies of *D. majalis*. The DNA data published by us in 2011 were typical of that technological period: 13 markers to characterise the plastids, and from the nucleus both the (justly) ever-popular nrITS and five nuclear microsatellite loci. We presented results for 15 English dactylorchid populations and remarkably, no less than eight of those populations failed to meet my prior expectations. In a salutary warning to my belief that I could identify even notoriously difficult dactylorchids by their appearance, the dactylorchid population occupying the Lancashire dune-slacks around Southport, which I regarded as a population dominated by *D. purpurella* with subsidiary *D. praetermissa*, proved to be the converse. However, the remaining problematic populations threw down a rather more substantial gauntlet.

Plastid genomes – increasingly recognised as being unreliable for circumscribing species due to “plastid capture” during hybridisation – proved unable to distinguish between *praetermissa* and *traunsteinerioides* (Figs. 1–7). But all seven of the questionable *D. traunsteinerioides* populations from southern and eastern England proved to clearly possess nuclear genomes that were instead typical of *D. praetermissa* (Figs. 8–14). These seven problematic sites, all of which occurred

Figs. 1–7: *Dactylorhiza traunsteinerioides traunsteinerioides* (1–4) and *D. traunsteinerioides francis-drucei* (5–7). 1 and 2, West Yorkshire; 3, North Yorkshire, 4, Anglesey; 5 and 6, Wester Ross; 7, Co. Antrim.  
Photos by Richard Bateman.