Phenological responses of British orchids and their pollinators to climate change: an assessment using herbarium and museum collections

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Abstract

Climate change might de-couple plant-pollinator relationships if species respond differentially to environmental cues, such as temperature, but studies have been hindered by lack of long-term data. This research validates natural history collections as a source of long-term phenological data and, using these data, investigates the phenological responses to temperature of flowering in British orchids and flight in their pollinators.

Herbarium specimens of *O. sphegodes* collected in the UK between 1848 and 1958 were compared to direct observation of peak flowering time in one population located in Southern England between 1975 and 2006. The response of flowering time to variation in mean spring temperature was statistically identical in both sets of data, providing the first direct validation of the use of herbarium collections to examine the relationships between phenology and climate. Using three important pollinator models: the solitary bee *Andrena nigroaenea* the digger wasp *Argogorytes mystaceus,* and the moth *Euclidia glyphica*, *m*useum specimens and field observation gave statistically identical results, confirming the value of museum collections as a source of long-term phenological data for insects.

For twelve of the fifteen orchid species studied, flowering advanced between 4.2 and 8.6 days for each 1°C increase in mean spring temperature, establishing phenological signals of flowering response to temperature. For all species mean monthly temperature in March, April or May was identified as a key temperature variable.

For the sexually deceptive orchid *O. sphegodes* there is considerable potential for a loss of synchrony between peak flowering time and peak flight of the primary pollinator, males of *A. nigroaenea* with further rises in spring temperature. The advancement in peak flight of the female bee with climate warming exacerbates the potential for disruption of pollination success.

Findings of this research reaffirm the need for detailed knowledge at species level in understanding the consequences of climate-driven phenological shifts for plants and their pollinators.

Key words: Central England Temperature (CET), climate change, flight time, flowering time, herbarium specimens, Hymenoptera, Lepidoptera, museum records, natural history collections, Orchidaceae, phenology

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Professor Tony Davy and Dr Dave Roberts: as my joint supervisors they have provided, in-depth guidance, ideas, and insightful comments to all parts of my thesis. They are co-authors on all manuscripts derived from this thesis.

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Chapter 1

Introduction

1.1 Background to the phenology of orchids and their pollinators in Britain: an assessment using herbarium and museum collections

1.1.1 Climate change and phenology

The rise in global surface temperature of approximately 0.74°C in the 100 years 1906-2005 is primarily attributed to the post-1976 period. Since 1976 the rate of increase has been approximately double that of the preceding period of warming, 1910-1945, and is more rapid than during any prior period within the last millennium (Walther *et al.* 2002; IPCC 2007). It is becoming increasingly apparent that the current period of climatic warming is driven, at least in part, by anthropogenic influences (Ulbrich & Christoph 1999; Parmesan 2001; Cook, Smith & Mann 2005; IPCC 2007). Within Britain, observed warming of mean annual Central England Temperature (CET) between 1956 and 2005 of 1°C is significantly above natural trend (HADCM3 control model) and is thought unlikely to be due solely to natural climatic variation (Karoly & Stott 2006). CET is the longest instrumental continuous surface temperature series available, with monthly mean values recorded in tenths of a degree from 1700 to date, and with continuous records from 1659 to present (Parker, Legg & Folland 1992; Parker & Horton 2005).

Ecological impacts of climate change across species embrace demographic (Pounds, Fogden & Campbell 1999), geographic (Parmesan 1996; Hill, Thomas & Huntley 1999; Thomas *et al.* 2001), altitudinal (Wilson *et al.* 2005) and phenological (Post *et al.* 2001a) effects. The interplay of diverse factors serve to impact on inter-trophic relationships and influence ecological interaction in wide-ranging and complex ways that are not easy to envisage (Both & Visser 2001; Penuelas & Filella 2001; Miller-Rushing *et al.* 2010). However an understanding of the ways in which individual species respond to climatic factors is an important step in recognising not only the potential threats to individual species but also to the integrity of species webs in the wider ecological landscape (Memmott 1999; Thackeray *et al.* 2010).

Phenological patterns reflect the timing of recurrent developmental or behavioural phases of organisms. The influence of temperature on the timing of key phenological

events has been widely reported for both flora (Fitter & Fitter 2002; Menzel *et al.* 2006) and fauna (Brakefield 1987; Fleming & Tatchell 1995; Crick *et al.* 1997; Woiwod 1997; Sparks, Jeffree & Jeffree 2000; Both *et al.* 2006). For temperate flora advancing phenologies are reported from Europe (Walkovszky 1998; Chmielewski & Roetzer 2001; Menzel *et al.* 2006; Bolmgren, Vanhoenacker & Miller-Rushing 2012; Holopainen *et al.* 2012), the Mediterranean region (Spano *et al.* 1999), North America (Bradley *et al.* 1999; Abu-Asab *et al.* 2001; Miller-Rushing & Primack 2008; Panchen *et al.* 2012) and Japan (Aono & Kazui 2008).

The interrelation of phenological phases which rely on environmental cues may become de-coupled as a consequence of rapid anthropogenic-induced climate change (Hughes 2000). This is especially so when species present differential temporal responses to environmental cues (Bradley *et al.* 1999; Thackeray *et al.* 2010). Whilst experimentation has demonstrated potential de-coupling of ecological interaction between trophic levels (Harrington, Woiwod & Sparks 1999; Petchey *et al.* 1999) there is little empirical data from field study. Much of the early phenological research has been concerned with the differential impact that climate change may exert between insect and food host-plant, such as bud burst and egg hatch of insect herbivores, and the implications for insect populations of asynchronous phenologies (Watt & McFarlane 1991; Buse & Good 1996; Watt & McFarlane 2002; Dixon 2003).

One phenological dimension which has been largely overlooked until relatively recently is the impact on plant-pollinator relationships (Memmot *et al.* 2007; Pradal, Olesen & Wiuf 2009; Rafferty & Ives 2011). The majority of plant species are pollinated by several or many pollinator species, often of diverse taxonomic origin (Herrera 1988). Apart from the Orchidaceae examples of obligate single-species pollination relationships are relatively rare (Bond 1994) and for most species there is the prospect that alternative species may replace those which are lost (Bascompte *et al.* 2003).

An experimental study of native plant species of North America did not flag mismatch of pollinator mutualisms (Rafferty & Ives 2011), whilst another North American study (Bartomeus *et al.* 2011) suggests that the average rate of advance of emergence of wild bees is broadly in line with the average advance of host-plant flowering, at least for

generalist species. However the direction and magnitude of response to temperature is highly variable and species-specific (Sparks, Jeffree & Jeffree 2000; Miller-Rushing & Primack 2008; Bolmgren, Vanhoenacker & Miller-Rushing 2012). To date there have been very few relevant studies reported in the literature (Bartomeus *et al.* 2011), none of which has examined specific plant-pollinator relationships. Patterns of flowering phenology are shaped in response to multiple selective forces and therefore would be expected to echo evolutionary compromises (Brody 1997). Even so spring flowering ephemerals would be expected to require close synchronicity with their pollinators (Abu-Asab *et al.* 2001) and highly evolved synchronicities, such as those repeatedly seen within orchid-pollinator mutualisms, may be particularly threatened.

Pollination success can be compromised by a range of factors including habitat loss, habitat fragmentation (Kearns, Inouye & Waser 1998), or invasion by non-native species (Kremen & Ricketts 2000) all of which may be linked to climate. Since time of pollination is a key factor in determining the timing of fruit set and subsequent seed dispersal, pollination success may not only have an impact on the plants own fecundity but also on that of diverse associated taxa dependent on pollen, nectar or seed resources of the host plant (Fitter & Fitter 2002). It follows that significant alteration in flowering phenologies have the potential to significantly disrupt ecosystem function (Memmot *et al.* 2007).

Three major environmental factors would be expected to influence flowering phenologies; temperature, photoperiod and precipitation (Rathcke & Lacey 1985) and within temperate zones it is clear that climatic factors play a critical role (Fitter & Fitter 2002). Whilst the factors contributing to pollination disruption are multifarious, potential threats posed by phenological asynchrony are of concern (Augspurger 1981; Post *et al.* 2001b; Parmesan 2006; Memmot *et al.* 2007; Thackeray *et al.* 2010). This research seeks to quantify species-specific phenology of temperate orchids in Britain using long-term data from natural history collection, and to examine specific plant-pollinator phenologies. Temperate British orchids represent ideal model systems with which to study phenological interactions for several reasons: they have been collected assiduously since the early 1800's, enabling long-term datasets to be derived for a range of species corresponding to detailed temperature records, they represent a range

of pollination systems from rewarding to food deceit and sexual deceit, and for several species the main pollinator species are known.

1.1.2 Datasets for phenological research

The impact of climate on phenology is species-specific and the key to understanding phenological patterns is held in long-term data (Fitter *et al.* 1995; Penuelas & Filella 2001; Sparks 2007; Bolmgren, Vanhoenacker & Miller-Rushing 2012). This is especially so because detection of phenological trends in relation to long-term climate change may be confounded by the influence of short term inter-annual or decadal climate variation arising from shorter-term circulation patterns (Badeck *et al.* 2004; Bolmgren, Vanhoenacker & Miller-Rushing 2012). Climate change studies, often with low signal-to-noise ratios, require sufficiently long-term data to elucidate any underlying trend. However the choice of species for long-term study of phenology has been dictated by the availability of suitable records. These are often scarce because of the overall shortage of long-term monitoring schemes (Sparks & Carey 1995).

Despite the instigation of phenology groups such as the European Phenology Network (EPN) (van Vliet *et al.* 2003; Menzel *et al.* 2006) for many species long-term data collected for the purpose of studying climate-induced phenological change are not available, or not easily obtained (Sparks 2007; Holopainen *et al.* 2012). Data collection is expensive and time-consuming with the result that datasets are often short-term or incomplete (Wolfe *et al.* 1987). The recent introduction of internet-based resources such as the UK Phenology Network established in 2000 and organised jointly by the Woodland Trust and the Centre for Ecology and Hydrology (CEH) http://www.naturescalendar.org.uk/ represents a valuable resource for promoting the relevance of phenology: gathering and collating data for a range of research projects. Whilst volunteer recorders provide valuable input there is inevitably an element of compromise between control over data quality and data coverage (Magurran *et al.* 2010). Recent phenological studies have utilised diverse sources of long-term data as an alternative to scarce field observation (Primack & Miller-Rushing 2012) including photographs (Miller-Rushing *et al.* 2006; Sparks, Huber & Croxton 2006; Crimmins & Crimmins 2008) and historical archives (Aono & Kazui 2008). But for many species the most readily available long-term data are held in natural history collections. The

Global Biodiversity Information Facility (GBIF), set up in 2001 http://www.gbif.org/ is a relatively new internet-based initiative which holds mainly natural history collections and facilitates access to these resources

1.1.3 The role of herbaria and museum collections

Historical data in the form of herbarium and museum collections are captured in three dimensions: physical entity, place and time. The vast number of collections worldwide –estimated to be in the order of 2.5 billion–and the increasing accessibility to data via the internet, makes this a data source with huge potential in scientific research worldwide (Graham *et al.* 2004). Whilst the potential contribution of these collections for current research had been largely overlooked (Suarez & Tsutsui 2004) until recently their relevance is increasingly recognised (Molnár *et al.* 2012). The use of natural history records as a source of biological data is increasingly being reported in the literature. For plants, information derived from herbarium specimens has been used to analyse changes in species distribution and abundance (McGraw 2001; Jacquemyn *et al.* 2005; Chauvel *et al.* 2006; Case *et al.* 2007), assess vulnerability under IUCN Red List Criteria (Willis, Moat & Paton 2003; Rivers *et al.* 2010), track patterns of disease ecology (Malmstrom *et al.* 2007), and investigate phylogeography (Saltonstall 2002). Similarly, herbarium and museum specimens have been used to reconstruct patterns of historic genetic variation (Cozzolino *et al.* 2007; Moritz *et al.* 2008; Rowe *et al.* 2011).

Herbarium records are unique in that they reflect an individual plant's phenological state at the time and location of collection, and therefore may represent an alternative for direct field observation. Recent studies suggest that herbarium collections can provide data that can be exploited in climate change studies, as reported trends have been broadly in line with phenological trends reported elsewhere in the literature (Sparks 2007) either as a multi-species average (Primack *et al.* 2004; Bolmgren & Lönnberg 2005; Miller-Rushing *et al.* 2006; Bowers 2007; Kauserud *et al.* 2008; Panchen *et al.* 2012) or as a single-species study (Lavoie & Lachance 2006). There has been, however, no direct validation of the efficacy of museum collections in phenological research as studies have relied heavily on comparison of averaged trends in order to corroborate results or have been used to demonstrate average shift toward

earlier collection. Different species do not respond uniformly to climate change (Sparks 2007) and although demonstration of averaged trends in flowering time can be insightful, herbarium collections have not been evaluated for their potential as proxy field data in the absence of long-term monitoring of individual species.

1.1.4 Plant- pollinator biology within the Orchidaceae

The Orchidaceae is a highly diversified family, with species inhabiting most regions of the globe and a wide range of ecological niches. There is some fluidity in the total estimated number of orchid species reported in the literature ranging between 19,500 (Jersáková, Johnson & Kindlmann 2006) and 25,000 (Nilsson 1992), but with total species numbers of such magnitude this is the most species-rich plant family.

The Orchidaceae represent a relatively recently evolved family in which individual species are now thought to have evolved after key pollinator groups had already established mutualisms with angiosperm species (Jersáková, Johnson & Kindlmann 2006). For many orchid species, evolutionary change is viewed as largely unilateral with little evolutionary impact on pollinator traits. This assessment is linked to the high incidence of non-rewarding pollination strategies within the family. Such strategies exploit existing plant-pollinator mutualisms and sexual behaviour in pollinating insects (Jersáková, Johnson & Kindlmann 2006) exerting no selective pressure on pollinators. The lack of reward for pollinators, as seen in deceptive orchid–pollinator interactions, results in fundamental asymmetry and thus co-evolution does not represent an appropriate evolutionary model.

Studies report infrequent visitation by pollinating insects, low fruit-to-flower ratios and low pollination rate (Darwin 1877; Ackerman & Mesler 1979; Nilsson 1983; Neiland & Wilcock 1998). These provide strong evidence that for many orchid species reproductive success is pollinator-limited and that optimal pollination is rarely achieved (Nilsson 1992; Neiland & Wilcock 1995; Tremblay *et al.* 2005). The amassing of pollen into pollinia is a central element in the patterns of dispersal of pollen and is a means of maximizing pollen dispersal under conditions of pollinator limitation.

The Orchidaceae are notable for the exceptional frequency of pollination mechanisms by deception and more than one third of orchid species are pollinated without offering reward to the pollinator (Schiestl 2005). Both its prevalence and repeated evolution within independent evolutionary lineages demonstrates a highly successful pollination strategy (Cozzolino & Widmer 2005). Food deception is the usual pollination strategy amongst species of the genera *Anacamptis, Dactylorhiza*, *Neotinea,* and *Orchis* (Scopece *et al.* 2007). In a study of 18 food deceptive Mediterranean orchid species six functional pollinator groups were established, although one third of orchids relied on pollinators within a single functional group. Hymenoptera represent 89% of all pollinators, demonstrating the importance of these aculeates (bees, wasps and ants) (Scopece *et al.* 2007). Lepidoptera (butterflies and moths) are also a significant pollinator group for European orchids (Claessens & Kleynen 2011).

1.1.5 Food deception

Orchids of food deceptive genera such as *Anacamptis*, *Dactylorhiza* and *Orchis* are typically grouped within one of two alternative modes of deception: firstly mimetic-specific to a particular co-blooming species (Batesian mimicry), or secondly as using a generalist deceit strategy (deception without model). The latter is considered the most common mode of food deceit (Johnson & Steiner 2000): here the orchid does not closely resemble any specific model species; rather it mimics co-flowering nectariferous flora in general. In this latter generalist mode plants may exhibit pronounced intraspecific floral variation (Johnson & Steiner 2000): naïve insect visitors eventually learn to avoid the non-rewarding plants and so floral variation may serve to prolong the period of deceit and enhance pollination success. Conversely, under Batesian mimicry variation in floral trait is constrained by the pattern of the model species.

 Flowering periods are frequently associated with the period of emergence of insect foragers, and optimum reproductive success is linked with the period during which inexperienced pollinating insects can be deceived (Nilsson 1992). This temporal association is especially important for early flowering species pollinated by newly emerged foraging bees, such as *Orchis mascula*.

Timing of flowering is important for food deceptive orchids: improvement in pollination success has been demonstrated when co-flowering with nectariferous magnet plants (Johnson *et al.* 2003). The overall effectiveness of food-deceptive strategies also relies on relatively low numbers of deceptive plants within a relatively large guild of rewarding plants (Scopece *et al.* 2007).

1.1.6 Sexual deception

The Orchidaceae is the only plant family in which pollination by sexual deceit is known (Dafni 1984; Nilsson 1992; Schiestl 2005; Tremblay *et al.* 2005). However recent research, demonstrating mating attempts by male bombyliid flies toward insect-like petal elements of the daisy *Gorteria diffusa* provide evidence of early stages in the evolution of sexual deceit outside of the Orchidaceae (Ellis & Johnson 2010).

To achieve sexual deceit the orchid mimics the female of the pollinator species, luring the male to attempt copulation, during which pollinia may become attached to the insect and transferred to another orchid, or received from another orchid. A combination of visual, tactile and chemical cues are used to entice the pollinator (Dafni 1984). Chemical mimicry of the female sex pheromone is the critical factor in attracting males over long-range (Schiestl 2005), whilst visual and tactile cues are used to stimulate pseudocopulation (Ayasse *et al.* 2000) . Modes of sexual deception have been most comprehensively studied in orchids of the European genus *Ophrys* which characteristically rely on pollination by male bees of the order *Hymenoptera* (Pedersen & Faurholdt 2007; Schiestl & Cozzolino 2008) . Within European orchids *Ophrys* is the only genus to rely on sexual deceit.

1.1.7 Orchid species within the UK

Some 56 orchid species are native to the British Isles (Harrap & Harrap 2005). Some of these are of Mediterranean origin, and populations in Britain are often toward the northern limits of their European range. Conversely there is a small number of northern temperate species with a southerly range limit across Britain.

For native British orchids soil preferences are the primary factor dictating habitat and ecological niche (Foley & Clarke 2005) so that habitat type can usually been assigned a distinct orchid flora. As a consequence of strict habitat requirements some species

are confined to a very restricted range and with isolated populations. A diverse series of habitat type is associated with British orchids; chalk grassland of the downlands of Southern England to acid marshlands, dune slacks and machair, ancient woodlands, wastelands and roadside verges (Foley & Clarke 2005; Harrap & Harrap 2005).

1.2 Thesis outline

1.2.1 Theme and Hypothesis

The underlying theme of this thesis is an investigation of the relationships between phenology and temperature for both plants and their insect pollinators. The central hypothesis of this research is that long-term climatic trends impact both the flowering phenology of plants and the flight phenology of pollinator species and that these long-term-trends will be detectable using natural history collections as a source of long-term data.

The central hypothesis of the thesis is expressed as two parts: firstly an examination of the value of herbarium and museum specimens as an alternative source of long term data for phenological research; and secondly an investigation of the relationship between climatic variables, flowering phenology of selected orchid species and the flight phenology of associated pollinators in Britain.

In temperate climates, characterised by distinct seasonality, developmental cues for many taxa may be temperature driven, and the effect of spring temperature is likely to be particularly significant (Aono & Kazui 2008; Amano *et al.* 2010). By quantifying changes in both plant and pollinator phenologies for specific species it may be possible to predict the potential impact of continued global warming on plant-pollination relationships. There may be divergent phenological effects if plants and their pollinators are differentially influenced by environmental cues. In such circumstances peak flowering times and peak flight times may become, to some degree, asynchronous. Orchid–pollinator relationships may be particularly susceptible to pollinator disruption since such associations can be highly specific, especially where pollination is achieved via sexual deception. Where the mode of pollination is non-rewarding, whether it be sexual deceit or food deceit, there is likely to be little convergent evolutionary pressure between plant and pollinator. For these species especially, where climate change is rapid, identification of species-specific

phenological trends may enable potential mismatch in plant -pollinator phenologies to be predicted.

1.2.2 Thesis aims

In order to develop the central theme of the thesis three general aims were addressed:

(1) to establish whether natural history collections in museums and herbaria serve as proxy for field observation, and to assess their limitations for the purpose.

(2) to use long-term data sets to analyse changes in flowering phenology of a representative range of orchid species in Britain and to relate these findings to species-specific ecology. Similarly long-term data are used to investigate the relationship between climate and the flight phenology of relevant pollinator species. Long-term data of three broad types was used; specimens held in herbaria and natural history collections, observational records held as elements of field studies, and climatic data recorded from meteorological stations. The aims were to identify key associations between climatic variables and flowering, to assess the degree of significance of potential explanatory variables, and to test the results against empirical evidence.

(3) to examine changes in orchid flowering phenology, and flight phenology of pollinating insects for explicit plant-pollinator relationships, and to consider what inferences can be made of the impact of future climate warming on these orchid-pollinator interactions.

The novelty of this research is threefold:

i) Investigation of the relationship between climate and peak flowering for a range of orchids in Britain has not been previously explored.

ii) Whilst natural history collections have previously been used as proxy for field data and as sources of data for phenological research their utility has not previously been validated. For the first time the value of herbarium and museum based specimens as proxy for field observation has been evaluated.

iii) Assessment has been made of the impact of climate on the phenologies of specific pollinator species in order to evaluate the potential for development of plant-pollinator asynchrony at a species level under continued climate warming.

1.2.3 Thesis structure


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│            Chapter 1                 │
│ Introduction and  background to the  │
│ phenology of orchids  and  their     │
│ pollinators. Outline  of thesis aims │
│ and hypothesis.                      │
└─────────────────────────────────────┘
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┌─────────────────────────────────────┐
│            Chapter 2                 │
│ An assessment of herbarium           │
│ specimens as  a source of            │
│ phenological data for climate change │
│ studies.                             │
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│            Chapter 3                 │
│ An assessment of  the phenology of   │
│ selected British orchids, using      │
│ herbarium data.                      │
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│    Chapter 4     │  │    Chapter 5     │
│ The phenology of │  │ The phenology of │
│ two sexually     │  │ both food-       │
│ deceptive orchids│  │ deceptive and    │
│ and their        │  │ food-rewarding   │
│ pollinators: an  │  │ orchids: an      │
│ assessment using │  │ assessment using │
│ museum and       │  │ museum and       │
│ herbarium data.  │  │ herbarium data   │
└──────────────────┘  └──────────────────┘
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┌─────────────────────────────────────┐
│            Chapter 6                 │
│ Summary of thesis findings and       │
│ overall conclusions.                 │
│ Discussion of the potential for      │
│ future work.                         │
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Figure 1-1. A schematic overview of the thesis, summarising the contribution of each chapter.

In **Chapter 2** the issue of whether herbarium specimens could represent a reliable proxy for direct field observation of peak flowering is addressed. First flowering can be an unreliable phenological data source and dates on which phenological stages reach their peak are preferable. However, such long-term field-based records are extremely rare. *Ophrys sphegodes* was used as the study species because it was the single orchid for which direct field observational data of peak flowering were available The field dataset was obtained from Professor Michael Hutchings at the University of Sussex and comprised a robust, contemporary 32-year population study of peak

flowering of *O. sphegodes* at a single site on the South Downs in Sussex between 1975 and 2006. This field-based dataset was compared to a long-term dataset constructed from historical herbarium specimens from the Natural History Museum, London (BM) and the Royal Botanic Gardens, Kew (Kew) collected over a 110 year period from 1848 to 1958.

In **Chapter 3** herbarium specimens held at the Natural History Museum, London (BM) and at the Royal Botanic Gardens, Kew (Kew) were used to construct phenological datasets for a representative range of British orchids. Fifteen species were selected representative of each of the three modes of orchid pollination: sexual deceit, (*Ophrys sphegodes*, *O. insectifera*), food deceit (*Anacamptis morio*, *A. pyramidalis*, *Cephalanthera longifolia*, *Dactylorhiza fuchsii*, *D. praetermissa*, *Neotinea ustulata, Orchis mascula*), and food rewarding (*Epipactis purpurata*, *Gymnadenia conopsea*, *Neottia ovata*, *Platanthera bifolia*, *P. chlorantha, Spiranthes spiralis*). The selected species also represented early, mid and late season flowering species and varying habitat type. This enabled flowering phenologies to be assessed across flowering period, pollination strategy and habitat type.

In **Chapter 4** flight phenologies of two Hymenoptera pollinator species are investigated using long-term data constructed from museum specimens held at the Natural History Museum, London (BM), Oxford University Museum of Natural History (OUM), and the University Museum of Zoology, Cambridge (UMZC). A secondary theme of this chapter is to validate the use of these entomological collections as proxy for field observation. Hymenoptera is an important pollinator group for several British orchid species including *O. sphegodes* and *O. insectifera*. The primary theme of this chapter is to investigate the phenological relationships between these two orchid species and their respective Hymenoptera pollinators (*Andrena nigroaenea* and *Argogorytes mystaceus*). Phenological interactions are examined and the potential for asynchrony between plant and pollinator is explored.

In **Chapter 5** flight phenologies of three Lepidoptera pollinator species (*Euclidia glyphica, Melanargia galathea, Deilephila elpenor*) are investigated from long-term data constructed from museum specimens held at the Natural History Museum, London (BM). A secondary theme of this chapter is to validate the use of these

entomological collections as proxy for field observation. Here museum records are compared directly to field observation and to relevant studies published in the literature. Lepidoptera are a major pollinator group for many orchid species in the British Isles. The primary theme of this chapter is an investigation of the comparative phenologies of selected British orchids (*Anacamptis pyramidalis*, *Platanthera bifolia*, *P. chlorantha*) and associated Lepidoptera pollinators, and an evaluation of the potential for asynchrony

The concluding remarks in **Chapter 6** summarise and discuss the results within the framework of the central theme of the thesis and places them in the context of related studies. Finally, proposed areas for the development of further work are outlined.

1.3 Orchid and pollinator species in this study

Native British orchid species included in this study are drawn from the Subfamily Epidendriodeae, tribe Neottieae (*Cephalanthera*, *Neottia* and *Epipactis*); Subfamily Orchidoideae, tribe Cranichideae (*Spiranthes*); and Subfamily Orchidoideae, tribe Orchideaea, subtribe Orchidinae (*Dactylorhiza*, *Gymnadenia*, *Platanthera*, *Orchis*, *Neotinea*, *Anacamptis* and *Ophrys*). Summaries of the life history of each of the fifteen species included in this thesis are outlined in chapters two and three.

Life histories of each of the selected insect pollinator species in this thesis are outlined in chapters four and five. These include two aculeates of the Order Hymenoptera, Superfamily Apoidea; Family Andrenidae, (*Andrena nigroaenea*); and Family Sphecidae, (*Argogorytes mystaceus*). Also included are the diurnal moth *Euclidia glyphica* (Burnet Companion), Family Noctuidae; the nocturnal moth *Deilephila elpenor* (Elephant Hawkmoth), Family Sphingidae; and the butterfly *Melanargia galathea* (Marbled White), Family Satyridae.

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Chapter 2

A validation of the use herbarium records as proxy for direct field observation: a case study with the orchid *Ophrys sphegodes*

An abbreviated version of this chapter has been published (full details can be found at Appendix 2).

Summary

1. The scarcity of reliable long-term phenological data has severely hindered study of the responses of species to climate change. Biological collections in herbaria and museums are potential sources of long-term data for such study, but their use for this purpose has lacked independent validation. This study represents a rigorous test of the validity of using herbarium specimens for phenological studies, by comparing relationships between time of peak flowering and climate derived from herbarium records and from direct field-based observations, for the terrestrial orchid *Ophrys sphegodes* in the UK.

2. Herbarium specimens of *O. sphegodes* collected in the UK between 1848 and 1958 were compared to direct observation of peak flowering time in one population located in Southern England between 1975 and 2006. The response of flowering time to variation in mean spring temperature (March-May) was virtually identical in both sets of data, even though they covered different periods of time which differed in extent and rate of anthropogenic temperature change. In both cases flowering was advanced by *c*. 6 days per 1°C rise in average spring temperature.

3. Predictions of peak flowering time based on the herbarium data corresponded closely with observed peak flowering times in the field, indicating that flowering response to temperature had not altered between the two periods over which the data were collected.

4. These results provided the first direct validation of the use of herbarium collections to examine the relationships between phenology and climate when field-based observational data are not available.

2.1 Introduction

Phenological events respond directly to climate. Recent climate change has undoubtedly affected the timing of development and seasonal events in many groups of organisms, including amphibians (Beebee 1995), birds (Crick *et al.* 1997), fungi (Kauserud *et al.* 2008) and plants (Sparks & Carey 1995; Fitter & Fitter 2002). Understanding the effects of recent climate change is a vital step towards predicting the consequences of future change. Moreover, only by elucidating the responses of individual species will we be able to predict the potentially disruptive effects of accelerating climate change on species interactions.

Detecting phenological trends in relation to long-term climate change is not straightforward. Since trends can be concealed by short-term inter-annual climate variation (Badeck *et al.* 2004), long datasets are needed. Despite the initiation of databases such as the European Phenology Network, for most species data collected specifically for the study of climate-induced phenological change are not available, or are difficult to find, reflecting the scarcity of long-term monitoring schemes (Sparks & Carey 1995). The choice of species for long-term studies of phenology has thus been dictated up to now by the availability of suitable field records. A further major obstacle is that most long-term data only record the beginning of phenological events in populations, such as dates of first flowering. Miller-Rushing, Inouye & Primack (2008) have shown that the use of such data to infer changes in phenology can be unreliable, and they advise that dates on which phenological stages reach their peak are preferable. However, long-term field-based records of the dates on which phenological events are at their peak are extremely rare.

Specimen-based records in biological collections are an alternate potential source of data, verifiable in both place and time, for the study of climate-induced phenological change. Until recently, the potential of such records has been largely overlooked (Suarez & Tsutsui 2004), even though the only data available for studying phenological trends in many species are those held in natural history collections in museums or herbaria. Recent phenological studies have utilised a range of less orthodox data sources, including historical archives (Aono & Kazui 2008), photographs (Miller-Rushing *et al.* 2006; Sparks, Huber & Croxton 2006; Crimmins &

Crimmins 2008) and herbarium specimens (Primack *et al.* 2004; Bolmgren & Lönnberg 2005; Lavoie & Lachance 2006; Miller-Rushing *et al.* 2006; Bowers 2007; Kauserud *et al.* 2008; Gallagher, Hughes & Leishman 2009). Herbarium records are unique amongst these alternative sources of information in that they represent a material specimen encapsulating an individual plant's phenological state at the time and location of collection, and therefore may represent a reliable substitute for field observation. Herbarium specimens are also likely to have been collected when phenological stages such as flowering are near their peak, rather than at an early or late stage in such seasonal events.

Recent studies undoubtedly suggest that herbarium collections may provide data that can be exploited in climate change studies, as findings have been broadly in line with trends reported in the phenological literature (Sparks 2007) and have supported the predictions of physiological models of phenological events such as flowering (Bowers 2007). Nevertheless, they depend on averaging-out the numerous possible influences and biases involved in a collection process that was not designed with the study of phenology in mind, within which the climatic signal-to-noise ratio might be low. Given the absence of long-term monitoring for most species, there is little direct evidence from which to evaluate the potential of averaged trends in events such as flowering time, derived from herbarium collections, as proxies for field data.

The aim of this research was to test the specific hypotheses *(i)* that flowering date would be advanced by warmer springs, *(ii)* that the relationship between flowering date and mean spring temperature would be the same in data derived from herbarium records and from field observations, and therefore *(iii)* that in the particular species for which this test was possible, herbarium records would be validated both as an effective proxy for long-term monitoring in climate-change research and as a predictor of phenological responses to future climate change.

2.2 Methods

2.2.1 The study species

Ophrys sphegodes Mill. Early Spider Orchid

Ophrys sphegodes (early spider orchid) is a species of southern and central Europe, with a northern range limit that includes southern England. It is associated with established, species-rich grassland over calcareous soils. The species is now rare in the UK, and largely confined to Dorset, West and East Sussex and Kent (Lang 1989; Harrap & Harrap 2005; BSBI 2011). The rapid decline in its range, which has been apparent in Britain since the 1930's, is attributed largely to habitat destruction through ploughing and altered grazing regimes (Fig. 2-1) (BSBI 2011).
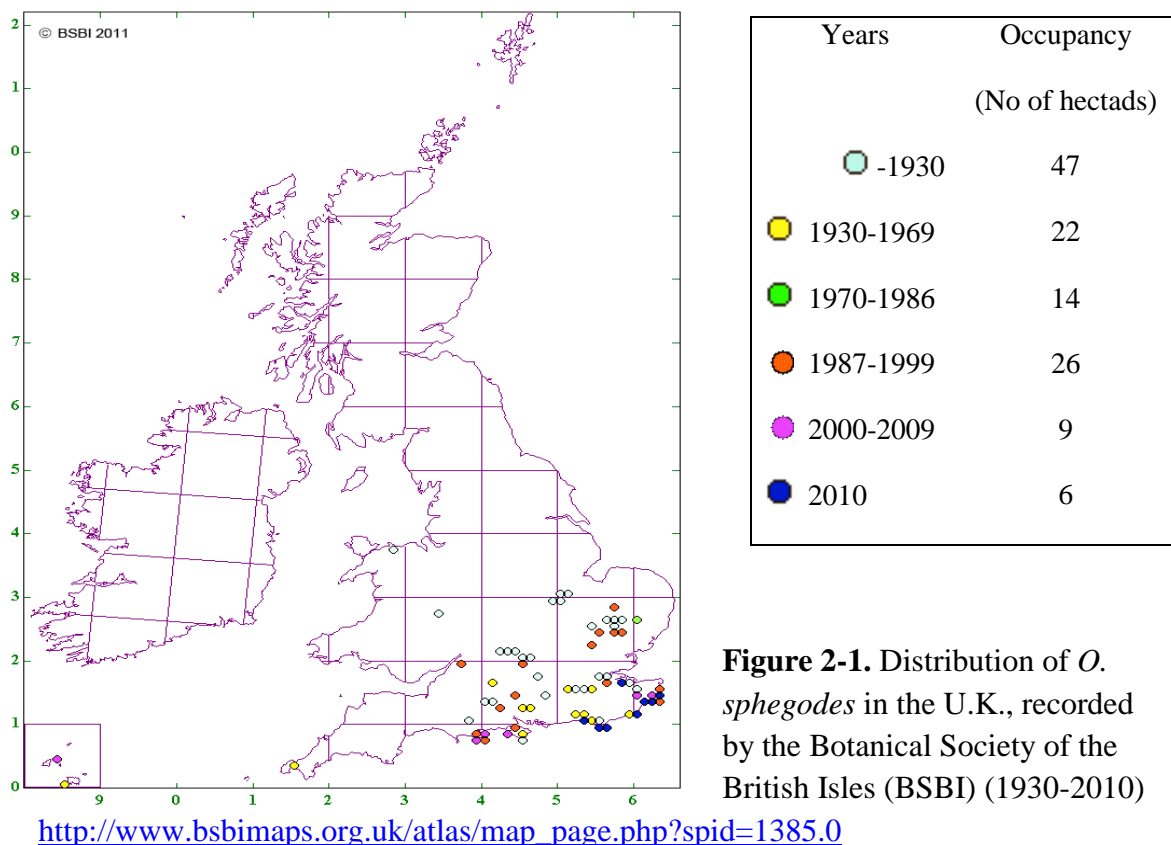
Years	Occupancy
	(No of hectads)
-1930	47
1930-1969	22
1970-1986	14
1987-1999	26
2000-2009	9
2010	6

Figure 2-1. Distribution of *O. sphegodes* in the U.K., recorded by the Botanical Society of the British Isles (BSBI) (1930-2010)

http://www.bsbimaps.org.uk/atlas/map_page.php?spid=1385.0

Although the length of the mycotrophic, subterranean phase of the life cycle of *O. sphegodes* is unclear (Hutchings 1989) it is a short-lived species after its first appearance above ground. Few plants survive for more than 10 years after initial

emergence (Hutchings 1987; Hutchings 2010) and rarely flowering for more than 3 consecutive years (Hutchings 1987). Most plants survive for less than three years (Hutchings 1989) and many individuals flower only once, and in their first year above ground (Hutchings 1987). In contrast to many orchid species which may spend several years in a vegetative state prior to first flowering (Wells 1981), the probability of *O. sphegodes* flowering in its first year above ground exceeds 0.7 (Hutchings 1987).The short life cycle makes the success of flowering, pollination and seed production highly important for population persistence (Hutchings 1987) . The importance of sexual reproduction is compounded by the low rate of vegetative propagation (Hutchings 1987). Seed set is reportedly low (Ayasse *et al.* 2000); rates of 6-18% have been recorded in Sussex, but higher rates have been reported for some populations in Kent (Lang 2004). Self fertilisation is inefficient, with low rates of seed production (Hutchings 1987).

In the UK the leaves of *O. sphegodes* usually emerge above ground in autumn (Hutchings 1989), forming rosettes of three or four leaves which attain full size by late autumn. These rosettes will overwinter, from which flower spikes emerge during April/May (April in Dorset, May in Sussex) (Hutchings 1987).

The flowering period is relatively short, commencing during late April or early May, and usually ending by late May (Lang 1989). In most populations in the UK inflorescences can bear up to six flowers but more plants bear four or fewer. Flowers open in succession from the base of the inflorescence, and most remain open simultaneously.

Pollination in *Ophrys sphegodes* is achieved as a consequence of sexual deceit of the males of the solitary bees *Andrena nigroaenea* (Schiestl 2005; Vandewoestijne *et al.* 2009). A combination of visual and chemical mimicry of the receptive female lure the male bee attempts to copulation, and in doing so pollen transfer from one flower to another may be achieved (Schiestl *et al.* 1997; Ayasse *et al.* 2000). However visitation numbers are not high and, since the female insects are more attractive to the males than the floral mimic (Nilsson 1992) it is likely that visitation rates fall once the females emerge. Pollination rates are generally low (Ayasse *et al.* 2000) and successful pollination is followed by rapid withering of the flower. Sanger and Waite (1998)

found that the number of inflorescences bearing ripening seed peaked at the end of June and that rapid dieback of the plant ensued. Flowering period is short irrespective of pollination success; few plants remain above ground at the end of July (Sanger & Waite 1998). This relatively short reproductive period would be expected to conserve any climatically-induced phenological signal.

2.2.2 *Herbarium data*

All 192 specimens of *O. sphegodes* held in herbaria at the Natural History Museum, London (BM, 133 specimens) and Royal Botanic Gardens, Kew (K, 59 specimens) were examined to verify identification (Fig. 2-2). Location, vice county, collector, habitat, and any notes on associated vegetation were recorded. Specimens with incomplete collection date were discarded All of the specimens originated from southern coastal counties of England (Dorset, Isle of Wight, Hampshire, East and West Sussex, and Kent), reflecting the sparse historical distribution of *O. sphegodes* (Fig. 2-3) (Carey & Dines 2002).

(a)   (b)

Figure 2-2. Representative herbarium specimens of *Ophrys sphegodes* held at (a) the Royal Botanic Garden, Kew (K) and (b) the Natural History Museum, London (BM)
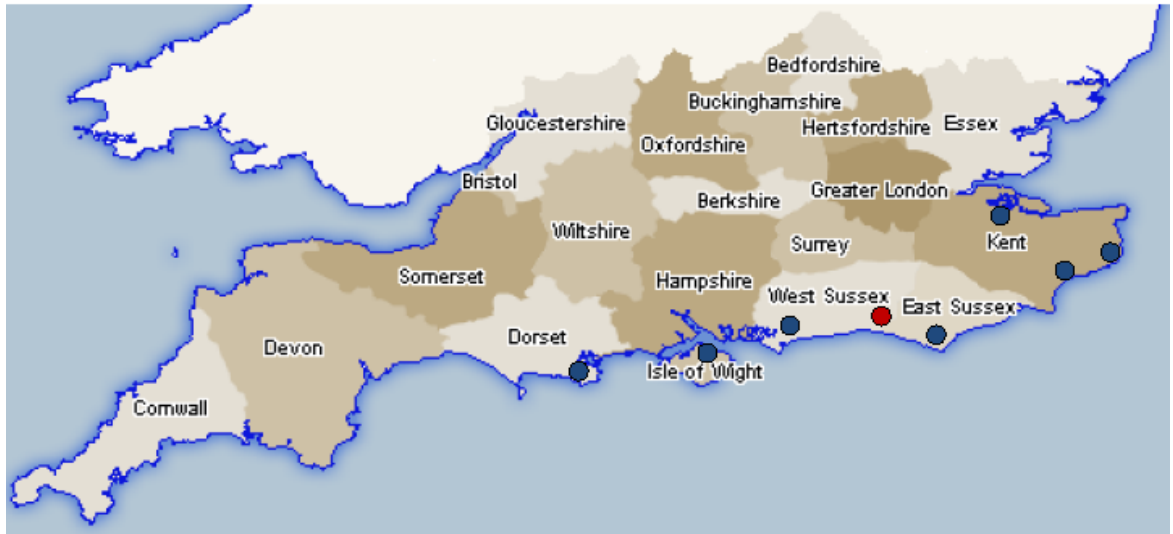
Figure 2-3. Map of southern England showing the location of Castle Hill Nature Reserve field study site, 1975-2006 (red); and all main locations of the herbarium specimens, 1848-1958 (blue).

Because of the rarity of *O. sphegodes* in the UK, the dataset was comparatively small and therefore it was important to ensure that the records represented the peak flowering stage as closely as possible. For each herbarium specimen total number of florets per spike, and stage of flowering (bud, open flower, senescent flower, and developed ovary) has been calculated and expressed as a percentage. Individuals in fruit or with senescent flowers were excluded. To ensure the dataset represented peak flowering, only specimens with at least 60% of their flowers open were included; whilst the flowers open sequentially from the base of the inflorescence normally most of the flowers become open at the same time in *O. sphegodes*. Some of the herbarium sheets consisted of multiple specimens mounted together. Where they had been collected by a single collector, on the same day and at the same location, they were treated as non-independent and the mean percentage of open flowers was derived.

Fifty three percent of the 192 specimens were rejected: 2 were damaged, 9 had unclear or illegible records of collection date, 31 were not dated, 60 were imprecisely dated (only the month or year), 3 were in seed, and 1 presented fewer than 60% of flowers open. Nine specimens were duplicates (multiple specimens) and therefore mean results

were used. The final data set comprised 77 specimens providing at least one data point for each of 57 years, spanning a 111-year period from 1848 to 1958. Collection activity was at its peak during the late 1800s and early 1900s (Fig. 2-4).
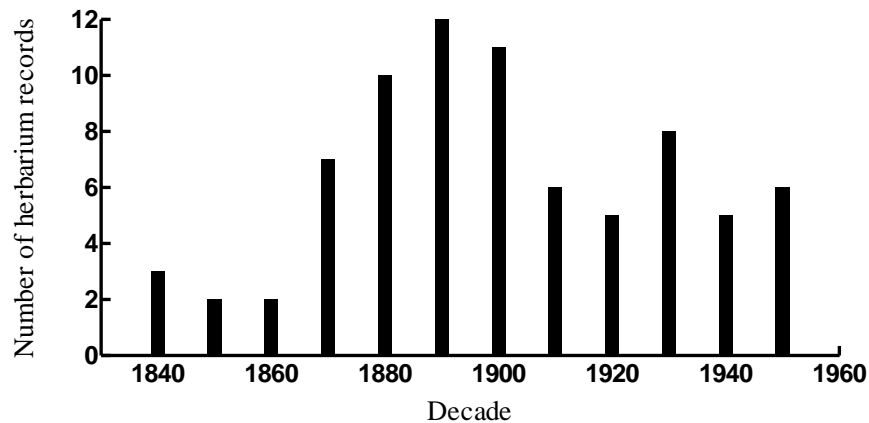
Figure 2-4. Distribution over time of dated herbarium records from the Natural History Museum (BM) and Royal Botanic Garden, Kew (K) for *Ophrys sphegodes* (years 1848-1958), *n = 77*

2.2.3. Field Data

Records of the peak flowering time of *O. sphegodes* were made in 25 of the 32 years between 1975 and 2006 in a demographic study of a population consisting of many thousands of plants at Castle Hill National Nature Reserve, East Sussex, UK (Hutchings 2010). The precise grid reference and co-ordinates of the study site (Fig. 2-5) are: grid reference TQ 376066; 50.842572 decimal degrees latitude; -0.046993 decimal degrees longitude. Peak flowering was based on assessment of the entire population to give a central tendency that would fit the flowering phenology of as many individual plants as closely as possible. This was done via inspection of flowering of the population at frequent intervals during the flowering period. Peak flowering was identified as the date when the population as a whole was at its height of flowering. This data was provided by Professor M.J. Hutchings of Sussex University, UK.

2.2.4.Meteorological Data

Mean monthly Central England Temperature (CET) records for the period 1848-2006 (Parker *et al*. 1992) were obtained from the UK Meteorological Office (http://hadobs.metoffice.com/hadcet/cetml1659on.dat). This is the only complete climate record available for the years during which the herbarium records and field data were collected. However, data for Central England are strictly representative only for a roughly triangular area enclosed by Bristol, Preston and London (Parker, Legg & Folland 1992). This is to the north of the distribution range of *O. sphegodes*. Three-month mean temperature data was calculated for each year (Winter- December (the prior year), January, February; Spring-March, April, May; Summer-June, July, August; Autumn-September, October, November).

Monthly mean temperatures were available from two Meteorological Office weather stations on the south coast, in locations corresponding with eastern and western centres of the distribution of *O. sphegodes*. Eastbourne, East Sussex UK, 21 km east of the Castle Hill field site, operated for the period during which the field records were collected.

Monthly minimum and maximum temperature data were available for Southampton, to the west, for all but 5 of the 111 years of the collection period covered by the herbarium specimens. Data from both of these collection sites would be expected to represent the climate within the distribution range of *O. sphegodes* better than the climate records available from CET. The means of monthly minimum and maximum temperature were used for both stations. Historical temperature data were not available closer to any of the sites of collection of the individual specimens in the herbarium records.

(a)

(b)

(c)

(d)

Figure 2-5. (a) Field Study Site; Castle Hill National Nature Reserve, East Sussex (Grid reference TQ 376066), and (b), (c), (d), *Ophrys sphegodes* in flower at this site on 28 April 2012

Mean monthly England and Wales's precipitation (EWP) figures for the period 1848-2006 were obtained from the Met Office website: http://www.metoffice.gov.uk/research/hadleycentre/CR_data/Monthly/HadEWP_act.txt. This was the only available historical record of precipitation to cover the whole of England.

Mean seasonal precipitation figures calculated for each year (Winter-December (the prior year), January, February; Spring-March, April, May; Summer-June, July, August; Autumn-September, October, November).

2.2.5 Geographical variation

The location of each specimen was initially assigned to vice county, and for those specimens where details of the collection site were recorded in sufficient detail, the actual coordinates of location site were calculated. Decimal latitude and decimal longitude were determined using the UK grid reference finder http://gridreferencefinder.com/index.php?lt=51.505505&lg=-0.07533989. Partial correlation analysis was conducted to separate the effects of latitude and longitude from temperature per se.

2.2.6 Analysis

The distribution of collection dates in the herbarium dataset for 1848-1958, were expressed as number of days after 1 April. The peak flowering date for the Castle Hill population in the years 1975-2006 was similarly expressed as days after 1 April. Both datasets were checked for normality and presence of outliers.

Both sets of flowering phenology data were examined for correlations with mean CET temperature data from the 9 months prior to the flowering season (i.e. the period of growth following breaking of tuber dormancy the previous summer). These data included mean monthly temperature and its averages over successive 3-month periods (September-November, December-February and March-May).

The period over which mean temperature and peak flowering date had the highest Pearson correlation coefficient (March-May) in both sets of data (see results) was designated 'spring'; multiple regression was used to investigate models of the dependence of flowering time on mean temperatures for different periods, resulting in

the adoption of mean spring temperature as the predictor variable (see results). In order to investigate the sensitivity of these models to the proximity of the temperature record, the analysis was repeated using Eastbourne mean spring temperature data for the field phenological regressions and equivalent Southampton data for the herbarium phenological regressions.

The linear regression model derived from the herbarium data and CET was used to predict peak flowering dates from mean spring temperature for the years between 1975 and 2006 for which field observations were available. Regression analyses were carried out with SPSS 16 (SPSS Inc., Chicago, IL, USA). Slopes and intercepts of regressions were compared via analysis of covariance (ANCOVA) (Zar 1984) using Graphpad Prism 5 (Graphpad Software Inc., La Jolla, CA, USA). Predicted flowering dates were compared with those actually observed using principal axis regression (Sokal & Rohlf 1969).

Both sets of flowering phenology data were also examined for correlations with mean EWP precipitation data from the 9 months prior to the flowering season (i.e. the period of growth following breaking of tuber dormancy the previous summer). These data included mean monthly precipitation and its averages over successive 3-month periods (September-November, December-February and March-May).

2.3 Results

Data derived from herbarium specimens over the 111-year period from 1848 until 1958, and recorded in the field between 1975 and 2006, both demonstrated the importance of spring temperature in determining flowering time. Significant correlations were found between peak flowering date and several measures of mean temperature in the CET records in the preceding months (Table 2-1).

2.3.1 Herbarium data

For herbarium material, there were significant correlations with mean temperature in March and May of the year of flowering but the highest correlation was with mean temperature over the 3-month period from March to-May (Table 2-1). No significant correlation was found between any measure of EWP and peak flowering time (Table 2-2).

2.3.2 Field data

Results for the field data were similar, but with significant negative correlations between peak flowering date and mean temperature for January, February, March, April and May (Table 2-1). The strongest correlation was again with the mean for the period March-May. There was also a significant negative correlation with mean winter temperature (December-February) but because of co-linearity between the variables, its addition to a multiple regression did not significantly improve the fit obtained with spring temperature alone. Inclusion of February mean temperature did not significantly improve the fit, and the inclusion of mean January temperature resulted only in a marginal improvement. Consequently, average temperature for March-May was the strongest basis for comparing herbarium- and field-based data for responses of flowering to temperature.

There was significant positive correlation with mean EWP for March prior to start of flowering and significant negative correlation with mean EWP for October of the autumn prior to flowering (Table 2-2). However, neither variable was included in a multiple stepwise forward regression model, indicating that, presumably due to co-linearity, neither variable significantly improved the fit in linear regression (Table 2-3).

Table 2-1. Comparison of correlations between flowering date and temperature for herbarium records and field data. Correlations are shown with mean temperatures for 3-monthly periods and individual months in the same year as flowering (January-May) or in the year previous to flowering (September-December). A negative correlation indicates that a higher mean temperature is associated with an earlier flowering date

Period of mean temperature	Herbarium data (1848-1958) $n = 77$	Field data (1975-2006) $n = 25$
Seasons:		
September-November	-0.004	-0.072
December-February	-0.065	-0.610**
March-May	-0.426**	-0.801**
Months:		
September	0.008	-0.273
October	0.108	0.226
November	-0.106	-0.171
December	0.047	-0.085
January	-0.003	-0.579**
February	-0.159	-0.549**
March	-0.396**	-0.609**
April	-0.153	-0.405*
May	-0.259*	-0.592**

Significance: ** $P < 0.01$; * $P < 0.05$

Table 2-2. Comparison of correlations between flowering date and precipitation for the herbarium records and the field data. Correlations are shown with mean precipitation for 3-monthly periods and individual months in the same year as flowering (January-May) or in the year previous to flowering (September-December). A negative correlation indicates that a higher mean precipitation is associated with an earlier flowering date

Period of mean precipitation	Herbarium data (1848-1958) $n = 77$	Field data (1975-2006) $n = 25$
Seasons:		
September-November (p.y.)	0.042	-0.345*
December-February	0.110	0.275
March-May	0.158	0.161
Months:		
September	0.055	0.090
October	-0.024	-0.536**
November	0.038	-0.132
December	0.144	0.409*
January	-0.012	0.020
February	0.051	0.069
March	0.077	0.465**
April	0.165	-0.200
May	0.054	0.053

Significance: ** $P < 0.01$; * $P < 0.05$

Table 2-3. Stepwise (forward) linear regression of field data (1975-2006), $n = 25$

	r	r^2	Std. error of estimate	F Change	Sig. F Change
Model 1*	0.609	0.371	5.329	13.571	0.001
Model 2**	0.740	0.547	4.625	8.540	0.008
Model 3***	0.840	0.706	3.816	11.317	0.003

Dependent variable: Field Data (1975-2006); Predictors:*March Temp: ** March, April Temp.;*** March, April, January Temp.

2.3.3 Comparing herbarium and field-based data for responses of flowering to temperature

As predicted, warmer years were associated with earlier flowering. The regression of flowering date obtained from the herbarium specimens on mean March-May (spring) temperature (Fig. 2-6a) accounted for 18% of the variation in flowering time. A 1 °C increase in mean temperature between March and May was associated with an advance in flowering of 6.5 days. Analysis of the field data yielded strikingly similar results. Linear regression of flowering date on mean spring temperature accounted for 64% of the variation in flowering time (Fig. 2-6b) and a 1°C increase in mean spring temperature was associated with an advance in flowering of 6.7 days. The regression models derived from the herbarium data and field data were statistically indistinguishable: neither the gradients ($F_{1,98} = 0.0035$, $P = 0.952$) nor the intercepts ($F_{1,99} = 0.0908$, $P = 0.764$) were significantly different, indicating that the phenological response to temperature was the same during the different periods over which the two sets of data were collected.
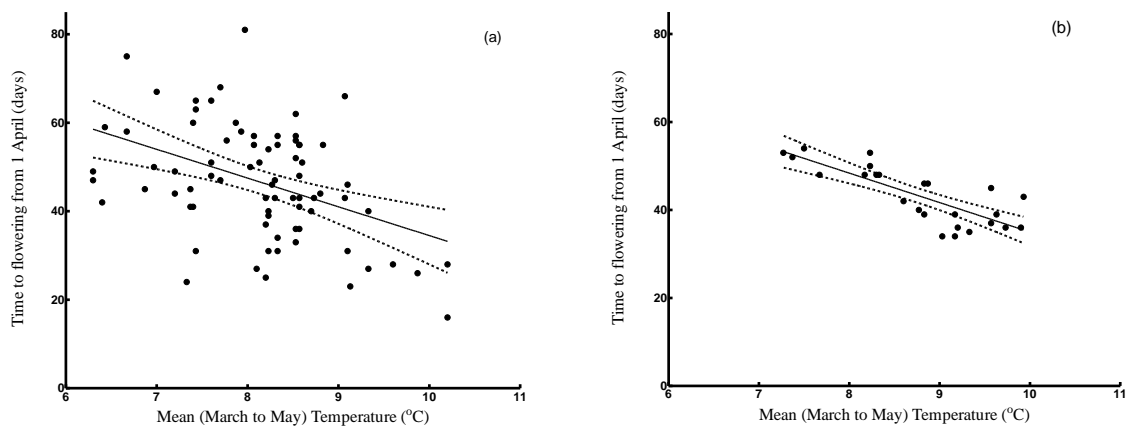
Figure 2-6. Relationships between flowering date (expressed as days after 1 April) and mean spring temperature (March-May) in Central England derived from (a) herbarium records from 1848 to 1958 ($y = 99.54 - 6.51\ x$, $r^2 = 0.182$, $P < 0.001$, $n = 77$) and (b) field data between 1975 and 2006 ($y = 101.88 - 6.69\ x$, $r^2 = 0.642$, $P < 0.0001$, $n = 25$)

2.3.4 Geographical effects

Applying the same analysis with more geographically appropriate temperature data for the field and herbarium records gave significant and strikingly similar results. Spring temperature at Southampton accounted for 13% of the phenological variation in herbarium data (Fig. 2-7a) and Eastbourne temperature accounted for 59% of that in the field data (Fig. 2-7b). In both cases flowering was advanced by 5.7 days per 1°C increase in spring temperature. The two regressions were again statistically indistinguishable (gradients, $F_{1,93} = 0.00007$, $P = 0.993$; intercepts, $F_{1,94} = 0.854$, $P = 0.358$). Furthermore, the gradients of the two regressions of field data on temperature, using Eastbourne and CET respectively, were not significantly different ($F_{1,46} = 0.481$, $P = 0.491$), and neither were the gradients of the two regressions with herbarium data using Southampton and CET temperature records, respectively, significantly different ($F_{1,145} = 0.130$, $P = 0.719$); this indicates that responses of the plants were consistent irrespective of the temperature records used. In both of these comparisons the intercepts were significantly different (field data, $F_{1,47} = 14.6$, $P = 0.004$; herbarium data, $F_{1,146} = 10.3$, $P = 0.002$), reflecting the differences between the temperature records used.
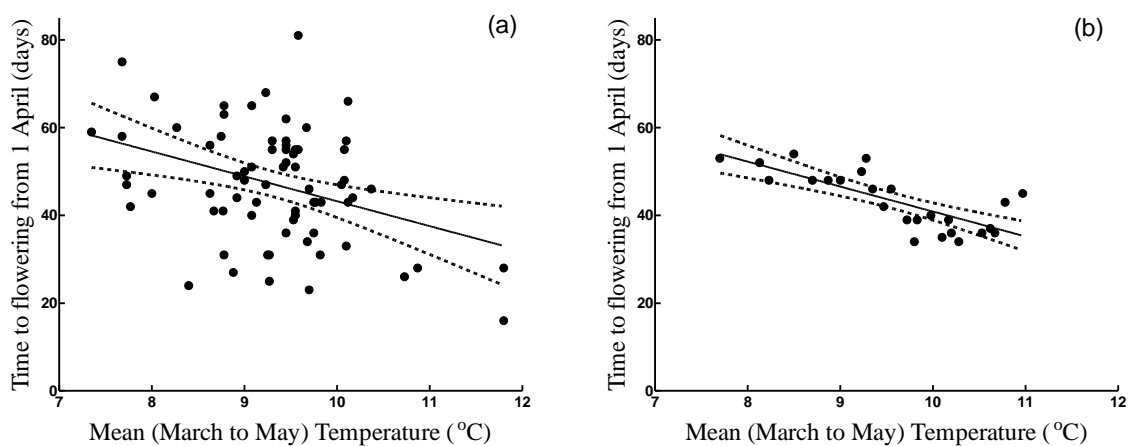
Figure 2-7. Relationships between flowering date (expressed as days after 1 April) and mean spring temperature (March-May): (a) between herbarium records from 1855 to 1958 and temperature at Southampton ($y = 99.8 - 5.66\ x$, $r^2 = 0.134$, $P = 0.0016$, $n = 72$); (b) between field data from 1975 to 2006 and temperature at Eastbourne ($y = 97.7 - 5.68\ x$, $r^2 = 0.586$, $P < 0.0001$, $n = 25$).

For the 69 herbarium specimens with sufficient geo-referencing to identify site location there were geographical ranges of 3.5 decimal degrees longitude and 0.8 decimal degrees latitude. These records were used to investigate the effect of longitude and latitude of origin on flowering time. Time to flowering was significantly correlated with both decimal longitude ($r = 0.468$, $P < 0.001$) and decimal latitude ($r = 0.247$, $P < 0.05$): this is as anticipated since flowering would be expected to be earlier with both westing and southing. Flowering was later at more easterly sites by an average of 4.86 days per degree longitude (Fig. 2-8a), and later at more northerly sites by an average of 12.17 days (Fig. 2-8b). However the apparent latitude effect was likely to be a spurious result of co-linearity between latitude and longitude ($r = 0.837$, $P < 0.001$): specimens tended to be repeatedly collected from a relatively few sites. The longitude effect represents climatic deviations around the underlying temperature trend expressed in the CET record. Each of the herbarium specimens was assigned to vice county of collection. Applying partial correlation analysis to separate the effects of latitude and longitude from temperature per se, the correlation between flowering date and mean spring temperature for the 77 herbarium specimens remained highly significant ($r = 0.326$, $P < 0.01$).
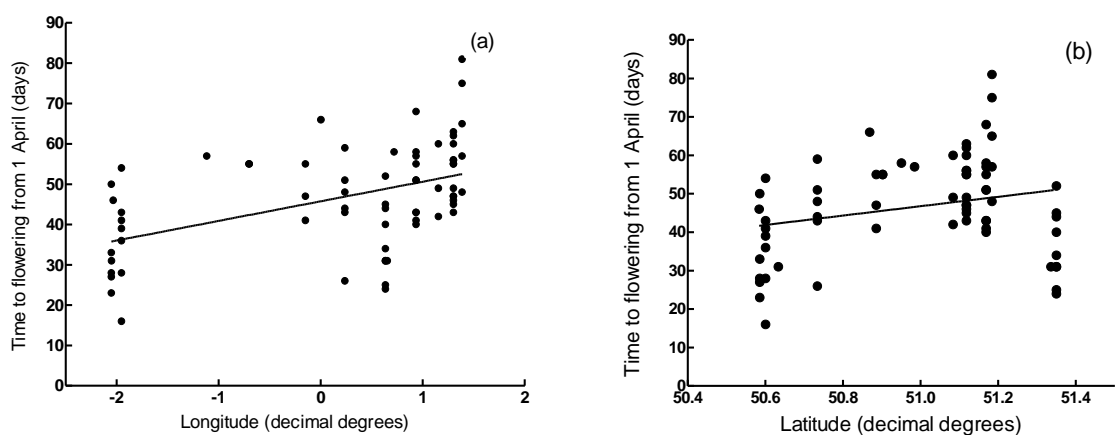
Figure 2-8. Relationship between flowering date (expressed as days after 1 April) and (a) longitude of collection site for the herbarium records ($y = 45.74 + 4.86$, $r^2 = 0.219$, $P < 0.0001$, $n = 69$), and (b) latitude of collection site (($y = -574.1 + 12.17$, $r^2 = 0.061$, $P < 0.05$, $n = 69$).

2.3.5. Using herbarium data to predict flowering phenology

The regression model derived from herbarium specimens (1848-1958) and CET was used to predict flowering dates for each of the 25 years between 1975 and 2006 for which there were field records of time of flowering. These predictions were highly correlated with the observed peak flowering dates ($P < 0.01$); the principal axis regression between observed and predicted dates had a coefficient close to unity (1.021) and accounted for 63% of the variation (Fig. 2-9a). The regression models derived from herbarium and field data was also plotted on the same graph to illustrate the extent of similarity (Fig. 2-9b).

(a)

(b)
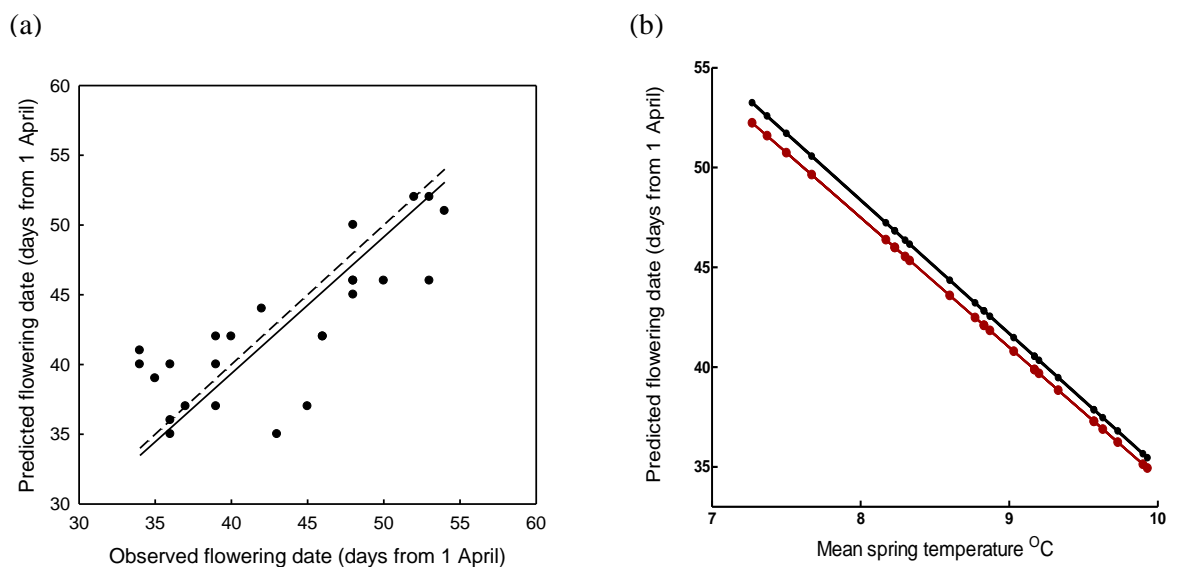
Figure 2-9. (a) Relationship between observed flowering date in the field (y_1) in 25 years between 1975 and 2006, and flowering date predicted from herbarium data for the same years (y_2). The principal axis regression (solid line) is $y_1 = -0.173 + 1.021 \, y_2$, $r^2 = 0.63$, $P < 0.001$, $n = 25$. The dashed line would apply if there were exact correspondence between the observed flowering date and the predicted flowering date. (b) Comparison of linear regression equations derived from herbarium (regression line coloured red) and field observation (regression line coloured black).

2.4 Discussion

Although biological collections can potentially provide valuable evidence of the impacts of climate change on the phenology of plant and animal species (Sparks 2007), their value as a proxy for field data has not previously been tested independently for any species. Miller-Rushing *et al.* (2006) compared flowering dates in recent benchmark years with those derived from historical photographs and herbarium specimens (1900-1921) for a range of species and found that not only were the deviations highly correlated with the corresponding differences in spring temperature but they yielded a trend that was very similar to that observed in independent field data of first flowering for the years 1887-1903. Bolmgren & Lönnberg (2005) established correspondence between flowering times derived from herbarium records and phenological observations, but did not investigate the underlying climatic drivers. The power of historical collection data to predict the consequences of future climate change needed to be tested directly.

The availability of field data for the rare terrestrial orchid *Ophrys sphegodes*, recorded at a single site in the UK over a 32-year period, provided a unique opportunity to validate the relationship between flowering date and mean spring temperature that was apparent from analysis of data from herbarium specimens collected over a much longer period. The comparison was greatly strengthened by the fact that peak flowering time was recorded in the field, rather than date of first flowering, which is more common in long-term phenological records. It is now clear that first flowering dates may not be ideal measures of plant responses to climate change, because the extremes of flowering distributions are more susceptible to confounding effects than central values (Miller-Rushing, Inouye & Primack 2008). Herbarium collections also tend to reflect peak flowering, as collectors generally aim to obtain prime specimens in full flower, as testified by the fact that only one specimen had to be discarded in which too few flowers were open to satisfy the sampling criterion.

Both historical and contemporary data showed that the peak flowering date of *O. sphegodes* was earlier in years with warmer springs, as expected (Hutchings 2010). This was the case both when phenological records were related to a common temperature record (CET) and when field and herbarium records were related to

different but more geographically focussed temperature records (Eastbourne and Southampton respectively).

The close correspondence between field and herbarium regressions, irrespective of the geographical locations of the temperature records tested, argues for the robustness of the relationships. The relationship with mean spring temperature remained highly significant using partial correlation analsysis to separate the effect of latitude and longitude from temperature for the herbarium data. Furthermore, using geographically different temperature records did not significantly alter the results for either contemporary or historical records.

Previous phenological studies have found similar correlations between flowering date and measures of spring temperature in spring- and summer-flowering species. The estimated advance in peak flowering date of 5.7 - 6.5 days per 1°C rise in temperature in *O. sphegodes* is within the range reported for advance in first flowering date in other species. Fitter *et al.* (1995) reported a mean advance of first flowering date of 4.4 days per 1 °C for 243 species at a single locality but with considerable differences between species; similarly, mean flowering dates of 24 species, averaged across the UK, advanced between 2 and 10 days per 1 °C (Sparks, Jeffree & Jeffree 2000).

The relationships between peak flowering date and spring temperature derived from contemporary and historical data for *O. sphegodes* were nearly identical, indicating a common response to spring temperature, notwithstanding that the historical collection and field observation periods were dissimilar in length, separated in time and different in geographical extent. This consistent response is important, as the pace of climate change has accelerated since 1975 when the field studies were initiated (IPCC 2007). None of the herbarium specimens was collected after 1958 and they therefore largely pre-date the period of fastest anthropogenic climate change.

Since the field and herbarium data were independent, it was possible also to test the power of the earlier herbarium records to predict the effects of subsequent climatic warming. Importantly, although there was some variation between years in the accuracy of predictions, the overall predictive power was extremely good, with the principal axis regression line for predicted and observed values lying close to the ideal 1:1 relationship.

Rigorous validation of the type presented here, although based to date on data for a single species, serves to increase confidence in the use of biological collections for predicting future phenological responses to climate change. Despite the strong underlying mean temperature signal, variation in flowering time may be influenced by a myriad of factors, and there are likely to be more confounding factors in the herbarium record than in the field data, because it includes specimens taken from a wider range of geographical locations and micro-habitats. Predictions based solely on mean spring temperature in Central England accounted for 18% of the variation in flowering date seen in herbarium specimens, but 64% of variation in flowering date in the field records from a single site. Use of more local temperature records in fact accounted for slightly (but not significantly) less variation in both cases, possibly because of the use of minimum and maximum temperatures averaged on a monthly rather than daily basis.

Despite the lower signal-to-noise ratio in the herbarium record, the signal was the same as in the field data and it was applicable over a much longer period. Bowers (2007) used physiological models based on previously determined flowering requirements (trigger dates and heat sums above a 10°C threshold) to predict, retrospectively, advancing flowering dates of shrubs in the Sonoran desert through the 20th century. A correlated tendency towards earlier collection dates in herbarium material over the same period supported the hypothesis that there had been a genuine response to changing climate, especially as there was no evidence that collector activity had changed over the period of study. However, the use of herbarium specimens assumes that they are representative samples of the population from which they are drawn.

The potential for bias resulting from variation in collection effort has been voiced as a concern by previous authors (Case *et al.* 2007). This study has demonstrated both that collector bias is not a problem when the herbarium data accepted for use in scientific studies are subjected to carefully controlled selection criteria, and that it is not necessary to have hundreds of specimens in order to extract useful information about the relationships between climate and time of flowering. However, although further validation using additional species with different phenologies is desirable, the extreme scarcity of suitable field observations limits opportunities for this to be achieved at the

present time. As a spring-flowering plant, *O. sphegodes* falls into a group identified as having flowering phenologies that are likely to be particularly sensitive to temperatures early in the year (Fitter *et al.* 1995).

Species that flower later in the summer may be less sensitive to warmer temperatures, and species that reproduce in the autumn may be sensitive in a converse sense; analysis of 34,500 dated herbarium records of autumn-fruiting of mushrooms in Scandinavia has revealed an average delay of 12.9 days since 1980, as the growing season has been extended by warming (Kauserud *et al.* 2008).

For most species of plants and animals, biological collections are the only source of long-term phenological data. It is estimated that some 2.5 billion specimens of flora and fauna are held in biological collections worldwide (Graham *et al.* 2004). The current drive toward digitisation of collections is facilitating the dissemination of the information they contain. An estimated 60 million records are already available for a wide range of taxa via internet information networks such as the Global Biodiversity Information Facility and HerpNET (Graham *et al.* 2004). With appropriate validation, the exploitation of this resource will have increasing relevance and value (Prather *et al.* 2004) for phenology studies which seek to understand and predict the consequences of continuing climate change.

In conclusion, this study represents the first rigorous validation of the use of herbarium specimens as proxy for field data, using the terrestrial orchid *Ophrys sphegodes* in the UK as a case study. Rigorous population studies are rare and the data from a one such study of a population at a site near the South Coast provided an exceptional opportunity to validate the method. The results of this study establish that the data derived from herbarium specimens have the capacity to be used for analysis, on an individual species basis, of long-term phenological trends. The current and continuing push to digitise herbaria and natural history collections, allowing far greater access to information and images, will be of substantial benefit to ecologists and other researchers seeking to understand the impact of climate warming on the phenology of individual species and species interactions. Natural history collections represent a key source of long-term phenological data for the majority of species of flora and fauna despite the activity of relatively recently established monitoring schemes such as the

European Phenology Network. In many cases this type of phenological data may be the only source available.

Chapter 2

2.5 References

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Chapter 3

The flowering phenology of selected British orchids: an assessment using herbarium specimens

Summary

1. Data derived from herbarium specimens collected over a period of 164 years (1823-1986) demonstrated the importance of spring temperature in determining flowering time for the orchid species in this study. Thirteen of the 15 orchid species (87%) showed a significant negative correlation with mean spring (March-May) temperature: warmer spring temperatures advanced flowering.

2. For 12 of the 15 orchid species (80%) in this study flowering advanced between 4.2 and 8.6 days for each 1°C increase in mean spring temperature. In stepwise forward regression mean monthly temperature in March, April or May was identified as a key variable for every orchid species irrespective of flowering time.

3. Spring and early summer flowering orchid species were most strongly correlated with temperatures over the three to four months immediately prior to flowering. For those species that flowered later in the season, seasonal temperature over spring and summer were equally important over the growing season.

4. In linear regressions of flowering time on mean spring temperature there were no significant differences among 12 (92%) of the 13 orchid species for which a test was possible.

5. Findings of a prior study (Molnár *et al*. 2012b) suggested that pollination mode may influence flowering response to temperature. In contrast, the hypothesis in this study that flowering response to temperature would be unaffected by pollination mode, reward or deceit, was supported by the results presented here.

3.1 Introduction

Flowering is considered one of the most significant phenological stages with which to evaluate sensitivity to climate change (Spano *et al.* 1999). This study investigates the relationship between climate and peak flowering time for 15 orchid species resident in the British Isles

Whilst field studies of orchid ecology have previously described how temperature and precipitation may be associated with variation in measures of plant performance, such as flowering frequency, year on year (Wells 1967; Wells 1981; Wells *et al.* 1998; Pfeifer, Heinrich & Jetschke 2006), only one prior study has examined flowering phenology in relation to climate. Hutchings (2010), in a 32-year study of *Ophrys sphegodes* (1975-2006) at a site in East Sussex, found that, on average, flowering advanced by 0.5 days per year^{-1} over the period of the study. Flowering was earlier after years with higher mean temperature in the year prior to flowering although the relationship was not quantified.

Whilst photoperiod can also be an important factor in flowering phenology many empirical studies, based on field observation, have demonstrated significant association between flowering phenology and temperature (Beaubien & Johnson 1994; Fitter *et al.* 1995; Sparks & Carey 1995; Walkovszky 1998; Abu-Asab *et al.* 2001; Menzel, Estrella & Fabian 2001; Fitter & Fitter 2002; Penuelas, Filella & Comas 2002). Geographic variation in flowering time would be expected for many plant species across Britain, reflecting both the latitudinal (North-South) temperature cline within the British Isles and the continental-oceanic (East-West) effect with warmer winters and cooler, wetter summers toward the west.

Examination of flowering phenology in relation to climatic or other environmental conditions is often based either on experimentation (Price & Waser 1998; Dunne, Harte & Taylor 2003; Llorens & Penuelas 2005) or long-term observational data (Fitter *et al.* 1995; Sparks, Jeffree & Jeffree 2000). Whilst long-term observational data may be preferred, especially when species are endangered (Tamm 1991) the scarcity of monitoring data has limited the opportunity for long term studies of flowering phenology (Sparks & Carey 1995). Recent studies have used herbarium specimens (Primack *et al.* 2004; Miller-Rushing *et al.* 2006; Gallagher, Hughes & Leishman

2009; Molnár *et al.* 2012b; Panchen *et al.* 2012) as an alternative source of 'field data' and their use as proxy for direct observation of mean flowering time has recently been validated (Robbirt *et al.* 2011). In a recent Hungarian study using herbarium data, flowering of the majority of Hungarian orchids had advanced over the last 50 years, by an average of 3 days, although the relationship with temperature was not quantified (Molnár *et al.* 2012b).

This study represents the first to use long-term data to investigate the relationship between flowering phenology and climate for a range of species within a single taxonomic group. The specific aim of this research was to use the extensive compilation of herbarium specimens collected in Britain since the early 1800s and held in herbaria of the Natural History Museum (BM), London and the Royal Botanic Gardens, Kew (Kew), to construct long-term data of peak flowering for a range of British orchids. The exceptionally comprehensive herbarium collections in Britain afforded the opportunity to examine more than 4,000 herbarium specimens, spanning 164 years between 1823 and 1986, for 15 species representing *c.*30% of the total number of orchid species present in the British Isles. The specific hypotheses were that *i)* the datasets would be sufficiently robust to establish phenological signals of flowering in relation to temperature, *ii)* peak flowering would be advanced with warmer temperatures prior to flowering and *iii)* using these herbarium collections, flowering phenology could be critically compared between species with differing flowering range and differing pollination mode. No difference in flowering response to temperature would be expected between orchid species, irrespective of pollination mode.

3.2. Methods

3.2.1 Herbarium data

Four thousand and forty six herbarium specimens held at Royal Botanic Gardens, Kew (K) and at the Natural History Museum (BM) were examined for the 15 orchid species in this study. Species were grouped by pollination mode: whether primarily pollinated by Hymenoptera, Lepidoptera or pollinator-generalists, and within this grouping, as rewarding or non-rewarding. Usable specimens spanned a collection period of 164 years between 1823 and 1986. (Fig. 3-1).Those specimens which were damaged, not

fully dated, without a location or illegible were discarded. Of those remaining the flowering stage of each specimen was assessed (according to criteria in Table 3-1) and for multiple specimens that were clearly collected by a single collector at the same location and date, the average stage of flowering was calculated. Those specimens with senescing flowers or which were in seed were discarded, as were specimens that were in bud or with less than 40% open flowers. This assessment of specimens was to ensure that only those that were flowering close to the peak flowering period were used in the datasets. Since the historic temperature data represented the temperature series for Central England it was desirable to confine the herbarium specimens to those collected from a geographically compatible region. Thus all specimens collected from Scotland, Wales, or the Isle of Man were rejected and neither were specimens examined from Ireland or the Channel Islands.

Table 3-1. The flowering stages to which herbarium specimens were assigned

Stage of orchid flower
In bud
Less than 20% open
21 - 40% open
41-60% open
61-80% open
81-99% open
100% open
Senesced flowers
In seed
Unknown stage due to damage or missing spike
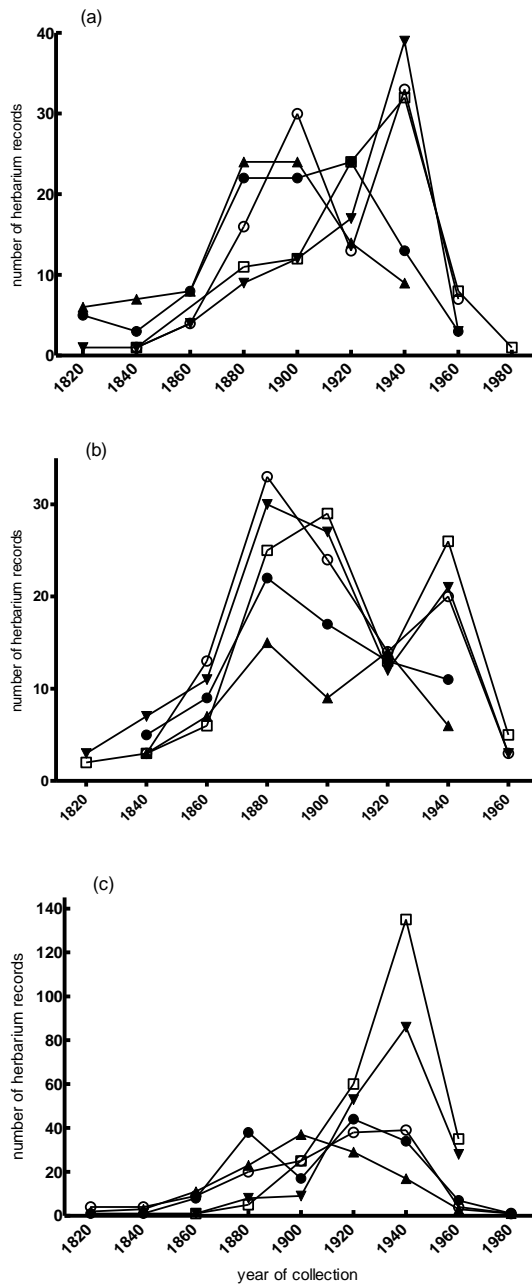
Figure 3-1. Collection over time of the 1,803 usable herbarium specimens (1823 – 1986), (grouped for ease of graphics) for; (a) ○ *E. purpurata*, □ *G. conopsea*, ▼*N. ovata*, ● *S. spiralis*, ▲ *N. ustulata*; (b) ○ *P. bifolia*, □ *P. chlorantha*, ▼*O. insectifera*, ● *O. sphegodes*, ▲ *C .longifolia*; and; (c) ○ *O. mascula*, □ *D. praetermissa*, ▼*D. fuchsii*, ● *A. pyramidalis*, and ▲ *A. morio*

3.2.2 The study species

3.2.2.1 Non-rewarding, Sexually Deceptive Orchids

3.2.2.1.1 Ophrys sphegodes Mill. Early Spider Orchid

A full description is provided in Chapter 2, section *2.2.1*. (See also Fig. 3-2c, d; Fig. 3-3a, b).

(a)

(b)

(c)

(d)
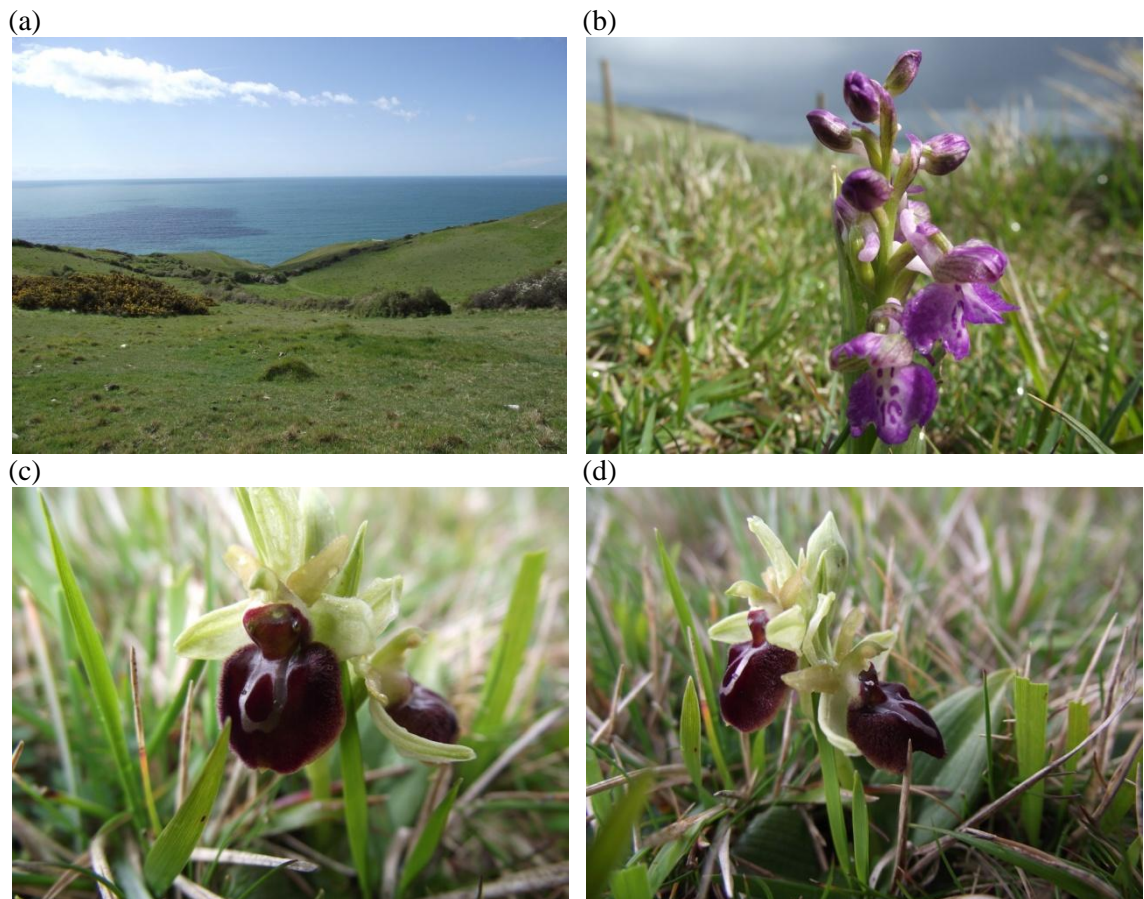
Figure 3-2. Langton Matravers, Dorset (SY 997783) 21 April 2012 (a): coastal site for *Anacamptis morio* (b) and *Ophrys sphegodes* (c) and (d).

3.2.2.1.2 Ophrys insectifera L. (syn. *Ophrys muscifera* Hudson 1761) Fly Orchid

The distribution range of *Ophrys insectifera*. is centred on the Mediterranean region but it also occurs across Western and Central Europe, and as far north as Scandinavia (Dorland & Willems 2002). It is widespread but uncommon in the British Isles, occurring sporadically as far north as Yorkshire and Westmoreland, and it is classified

as vulnerable in the 2005 Red Data List for Vascular Plants for Great Britain (Cheffings *et al.* 2005). The majority of populations are clustered across southern counties; especially on the chalk grasslands of Kent, Sussex and Surrey. Despite favouring calcareous soils, habitat is very varied: open woodland, particularly beech (Fig. 3-4c, d), alkaline fenland and wet margins of seasonal lakes. Within the NVC system the species is associated with calcareous grassland (CG), woodland (W12) and mire (M13) communities (Rodwell 1991b; Foley & Clarke 2005). In Britain populations have been recorded to 390 m above sea level (Pearman & Corner 2003), Flowering period is from late April to early July, with a peak in May and June (Harrap & Harrap 2005).

(a)　　　　　　　　　　　　　　　　　(b)
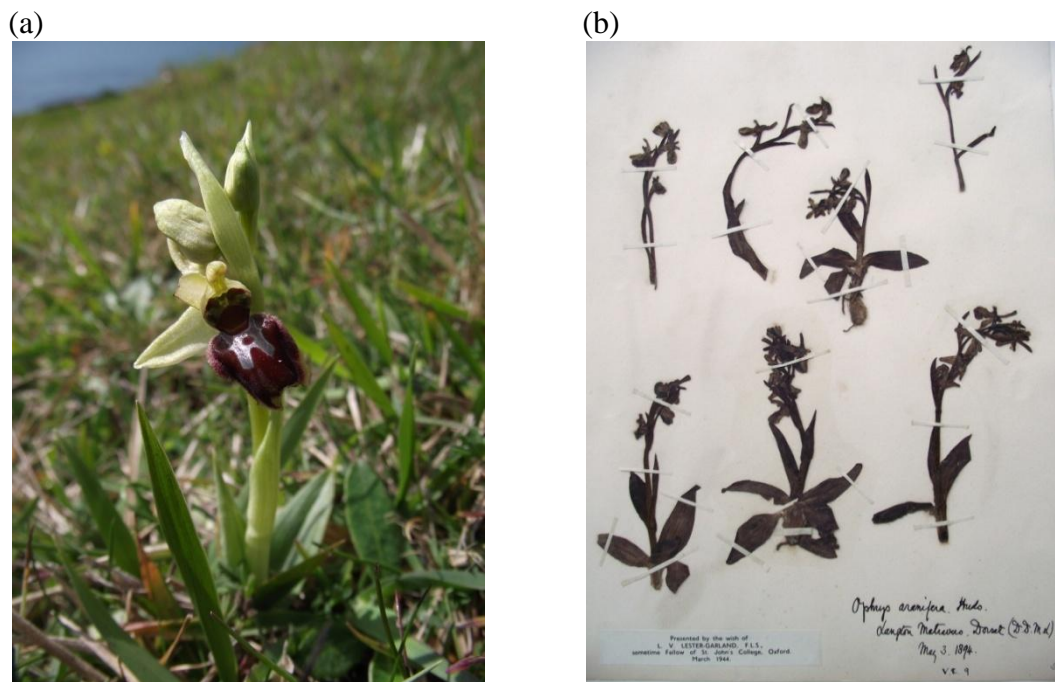
Figure 3-3. *Ophrys sphegodes* in flower at Langton Matravers, Dorset on 21 April 2012 (a) and as a herbarium specimen collected from Langton Matravers, Dorset on 3 May 1894 (b) and held at the Herbarium of the Royal Botanic Garden, Kew (K)

Most pollination is as a result of sexual deceit of the male digger wasp *Argogorytes mystaceus* (van der Pijl & Dodson 1966; Borg-Karlson 1990; van der Cingel 1995; Allan 2001; Claessens & Kleynen 2011), although the slightly later emerging *A. fargei*

(van der Cingel 1995; Claessens & Kleynen 2011), which is rare in the UK, is a subsidiary pollinator. 'Pollination rates' in the British Isles are reported to be less than 20% (Summerhayes 1968; Harrap & Harrap 2005), and it is probable that this refers to fruit set as a proportion of the flowers or plants.

(a)

(b)

(c)

(d)

Figure 3-4. Chapetts Copse, Hampshire (SU 654230) 15 May 2011: established beechwood site (a) for *Cephalanthera longifolia* (b), *Ophrys insectifera* (c) and (d).

3.2.2.2 Non-rewarding, Food Deceptive Orchids

3.2.2.2.1 Orchis mascula (L.) L. Early Purple Orchid

Orchis mascula is described as a Euoceanic species since its distribution favours the north and west of Europe (Kretzschmar, Eccarius & Dietrich 2007). In the Alps it is recorded at altitudes of up to 1800 m (Kretzschmar, Eccarius & Dietrich 2007) and to 880 m in the British Isles (Pearman & Corner 2003).

The species is widespread throughout Britain including the Hebrides, Orkney, Shetland, Isle of Man and Channel Islands although largely absent from parts of western Wales (Harrap & Harrap 2005). Whilst not a common species it can be locally abundant in favoured habitats: partcularly associated with calcareous grassland (Fig. 3-5b) and pasture, open downland, coastal cliffs, hedgerows, sand dunes, roadside verges (Foley & Clarke 2005), and woodland habitats (Kretzschmar, Eccarius & Dietrich 2007; Jacquemyn *et al.* 2009). Classified within mesotrophic grassland communities of NVC *Anthoxanthum odoratum- Geranium sylvaticum* (MG3), *Dryas octopetala-Carex flacca* heath (CG13), and *Luzula sylvatica-Geum rivale* tall herb communities (U17) (Rodwell 1992).

In Britain onset of flowering usually occurs during April and peak flowering time is between late April and late May. Flowering period is slightly later in northern and upland regions, on occasion lasting into early July (Harrap & Harrap 2005).

The flowers offer no nectar reward, although a sugary sap may be exuded within the spur, and are primarily pollinated by bees foraging for nectar after emerging from hibernation in spring. The mode of deception is one of generalised food deceit since the orchid does not mimic a specific species. Bees within the family Apidae have been named as pollinator species: principally *Bombus* species (Darwin 1877; Cozzolino *et al.* 2005; Kretzschmar, Eccarius & Dietrich 2007; Claessens & Kleynen 2011). In a seven-year Swedish study across eight locations the majority of pollinia were observed to be transported by *Bombus* queens, *Psithyrus* females and bees of the species *E. longicornis* (Nilsson 1983a). Successful pollination may rely on flowering when *Bombus* queens and *Psithyrus* females emerge from hibernation (van der Cingel 1995).

3.2.2.2.2 Anacamptis pyramidalis (L.) Rich. Pyramidal orchid

Anacamptis pyramidalis has a broad distribution across Europe, being widespread across Central and Southern Europe and the Mediterranean region, (Foley & Clarke 2005; Kretzschmar, Eccarius & Dietrich 2007), also parts of Russia and neighbouring countries (Vakhrameeva *et al.* 2008; Shetekauri & Jacoby 2009).

The occurrence of *A. pyramidalis* is fairly widespread across England, Wales and Ireland (Preston, Pearman & Dines 2002), where it is restricted to sea level and lowland areas up to *c.*300 m (Wilson 1956). In England it is most frequent in the south and east and becomes rarer in the west and north. In Scotland it is scarce and largely confined to the Western Isles, and western coastal regions (McNeill 1910; Clapham, Tutin & Moore 1987; Pankhurst & Mullin 1991; Preston, Pearman & Dines 2002), rarely reported along the south-eastern coast of Scotland (Preston, Pearman & Dines 2002). Peak flowering period is late June to mid July (McNeill 1910; Summerhayes 1968).

Across Britain it is essentially a species of chalk grassland (Fig. 3-5d) and its primary NVC classifications refer to calcareous grassland communities (CG1-CG6), also mesotrophic grasslands (MG1, and MG5), sand dunes (SD7, SD8, SD9, SD15b and SD18) and occasionally woodland communities (W6d and W21d) (Rodwell 1992; Foley & Clarke 2005).

Pollination is effected by a range of Lepidoptera: including butterflies, plus day-and night-flying moths: Fifty seven species of Lepidoptera have been reported carrying pollinia of *A. pyramidalis*, including burnet moths (Zygaenidae), the burnet companion *Euclidia glyphica* and the Marbled White butterfly, *Melanargia galathea* (Darwin 1877; van der Pijl & Dodson 1966; Proctor & Yeo 1973; Arditti 1992; Claessens & Kleynen 2011).

Figure 3-5. Noar Hill, Hampshire (SY 740320) 14 June 2008 (a) chalk grassland site for; (b) *Orchis mascula* 5 May 2008, (c) *Dactylorhiza fuchsii* 14 June 2008 and (d) *Anacamptis pyramidalis* 14 June 2008.

Rates of fruit set are variable: Lind *et al*.,(2007) recorded rates between 15 and 74% for different populations on the island of Öland, whilst Neiland & Wilcox (1998) found lower rates (33%) amongst populations in England.

3.2.2.2.3 Anacamptis morio (L.) R.M. Bateman, Pridgeon & W.M. Chase. (syn. *Orchis morio* L.) Green-winged orchid

Anacamptis morio is fairly widespread across Central and Eastern Europe, North Africa, Turkey and parts of the Middle East (Harrap & Harrap 2005; Kretzschmar, Eccarius & Dietrich 2007). Distribution in Britain centres across south eastern and central England, coastal regions of south and south west England (Fig. 3-2a, b) and Wales. (Lang 2004; Harrap & Harrap 2005). It is typically associated with short, unimproved grassland over calcareous or neutral soils and can be locally abundant in preferred habitats of damp unimproved meadow and old species-rich calcareous grassland. Its primary NVC classification is within the *Cynosurus cristatus-Centaurea nigra* grassland community (MG5) (Rodwell 1992). It is essentially a lowland species and maximum elevation in the British Isles has been recorded at 300 m (Wilson 1956).

The species is principally pollinated by foraging Hymenoptera. *Bombus* queens act as primary pollinators (van der Cingel 1995; Johnson, Craig & Ågren 2004), and some thirteen species have been identified (Darwin 1877; Nilsson 1984; Smithson 2002; Cozzolino *et al.* 2005; Claessens & Kleynen 2011). No nectar is produced, and the mode of pollination has been described as 'generalized food deception' since the orchid does not mimic a particular rewarding species. Instead it relies on co-flowering rewarding plants to serve as magnets for foraging bees. The sweet scent produced by *A. morio* is sufficient to deceive naïve bees, newly emerged from hibernation, into foraging amongst the orchid flowers (Johnson, Craig & Ågren 2004).

3.2.2.2.4 Dactylorhiza fuchsii (Druce) Soó (syn. *Orchis fuchsii* Druce, *Dactylorhiza maculata* subsp. *fuchsii* (Druce) Hyl, *Dactylorchis fuchsii* (Druce) Verm.) Common Spotted Orchid

Widespread across Europe, including Mediterranean regions, Scandinavia, Russia and Siberia, *Dactylorhiza fuchsii* is the most common and widespread orchid in the British Isles. It occurs throughout much of Britain and Ireland and across a range of habitat including grassland (Fig. 3-5c), open woodland, wet meadows, fens (Fig. 3-6c), machair and dune slacks. Whilst favouring calcareous or neutral soils it also occurs in mildly acidic heath or moorland (Harrap & Harrap 2005). Favoured communities are

mesotrophic grasslands (MG3, MG9) and grasslands of *Festuca ovina-Carlina vulgaris* and *Bromus erectus* (CG2-3) (Rodwell 1992).

Flowering occurs between mid May to early August (Lang 2004; Harrap & Harrap 2005). No free nectar is produced (Darwin 1877) and the species is generally considered non-rewarding.

Pollen analysis has established that the species is visited by a wide range of pollinating insects (Neiland & Wilcock 1999). Primary pollinators are Coleoptera; less frequently Diptera and Hymenoptera (van der Cingel 1995; Lang 2004; Claessens & Kleynen 2011). Seed set is reportedly high (50-90%), especially in the lower flowers which are first to open.

3.2.2.2.5 Dactylorhiza praetermissa (Druce) Soó (Syn. *Orchis praetermissa* Druce, *Dactylorchis praetermissa* (Druce) Verm.) Southern Marsh Orchid

This species is restricted to the Atlantic fringe of North West Europe (Foley & Clarke 2005; Harrap & Harrap 2005). In Britain it is most frequently seen in the south and south-east, southern coastal regions of Wales, but is rare in northern areas. Damp calcareous habitats are preferred, typically fenland (Fig. 3-6d) and marsh and principal communities are mire (M9-13, M22-24), swamp (S24) and dune slack (SD14-16) (Rodwell 1991b; Foley & Clarke 2005). In mildly acidic conditions populations are relatively small. It is essentially a lowland plant, and rarely occurs above 200 m. Flowering period is typically late May to early July, rarely to August.

There is little documentation of pollinator species: named species are *Trichius fasciatus* (Coleoptera) and *Eucera longicornis* (Hymenoptera) (Claessens & Kleynen 2011). Hybridization does occur with the co-flowering *D. fuchsii*, hence pollen vectors are likely to be comparable.

(a)

(b)

(c)

(d)

Figure 3-6. Noar Hill, Hampshire (SY 740320) chalk grassland site for (a) *Gymnadenia conopsea* 4 June 2012 and (b) *Neottia ovata* 4 June 2012; Cothill Fen Nature Reserve, Oxfordshire (SU 467995) fenland site for (c) *Dactylorhiza fuchsii* 8 July 2012; Wicken Fen National Nature Reserve, Cambridgeshire (TL 562705) fenland site for (d) *D. praetermissa* 11 July 2012.

3.2.2.2.6 Cephalanthera longifolia (L.) Fritsch (syn. *Cephalanthera ensifolia* Rich., *Epipactis ensifolia* F.W.Schmidt, *Serapias longifolia* (L.) Scop.) Sword-leaved Helleborine

This is essentially a European species with a broad latitudinal range from the Mediterranean to Scandinavia and Southern Finland. Preferred sites are open areas of woodland, especially beech, or mixed ash and oak, over calcareous substrates, and it is classified primarily within the *Fagus sylvatica-Mercurialis perennis* woodland community (W12) (Rodwell 1991a). Scattered sites occur in England, western Scotland and Wales but strongholds are Argyll and Hampshire. In the UK *C. longifolia* is classified as Vulnerable in the 2005 Red Data List for Vascular Plants (Cheffings *et al.* 2005). The largest population in the UK is at Chapetts Copse in Hampshire (SU 654230), a beechwood site hosting a population in excess of 2,000 plants (Fig. 3-4a,b) (Harrap & Harrap 2005; Rumsey 2010). Flowering period is mid May to mid June, slightly later in Scotland.

The species is pollinated by Hymenoptera: bees of the genera *Halictus, Bombus* and *Lasioglossum* (Godfrey 1933; Summerhayes 1968; Claessens & Kleynen 2011). Flowers are non-rewarding and pollination success relies on co-occurring magnet plants: the orchid does not mimic a particular species, rather it exploits facultative floral mimicry within a generalized food deceit system (Dafni & Ivri 1981; Hedley 1999; Rumsey 2010). Plants are capable of self pollination, however selfing is not routine given the low rates of seed set, and pollination is presumably limited by the low numbers of visiting insects (Proctor & Yeo 1973).

3.2.2.2.7 Neotinea ustulata (L.) R.M. Bateman, Pridgeon & M.W.Chase (syn. *Orchis ustulata* L.) Burnt-tip Orchid

This species is found throughout much of Europe, where it occurs as far north as the Faroes, Scandinavia, the Urals of Russia and western Siberia. Southern range limits pass through Spain, Italy, northern Greece and the Balkans. Once found throughout the calcareous regions of England, it has suffered a severe decline over the last 70-80 years. Populations are now localised and largely confined to protected areas and established, undisturbed chalk grassland communities (CG2) (Rodwell 1992) of southern England although a few populations remain in the north. The decline in range

is considered to be a result of habitat disturbance; altered grazing regimes and changes in agricultural techniques (Tali, Foley & Kull 2004).

Two varieties are recognised: var. *ustulata* is the more common and flowers from mid May to early June, while variety *aestivalis* flowers during July and August (Tali, Foley & Kull 2004). There is little difference in morphology between the two varieties: subtle differences in labellum shape and markings which are not wholly consistent in British populations (Tali, Foley & Kull 2004).

Neotinea ustulata is scented but produces no nectar reward (Tali, Foley & Kull 2004; Kretzschmar, Eccarius & Dietrich 2007). Information about pollinator vectors is scant (Tali, Foley & Kull 2004): tachinid flies *Echinomya magnicornis* (Diptera) and the longhorn beetle *Leptura livida* (Coleoptera) are named pollinators (van der Cingel 1995; Tali, Foley & Kull 2004; Claessens & Kleynen 2011).

3.2.2.3 Rewarding Orchids

3.2.2.3.1 Gymnadenia conopsea (L.) R. Brown. (syn *Habenaria conopsea* (L.) Benth.*, Habenaria gymnadenia* Druce*, Orchis conopsea* L.) Chalk Fragrant Orchid

Relatively recent taxonomic revision of *Gymnadenia conopsea s.l.* has resulted in the separation of the former complex of three subspecies into three distinct species: *G. conopsea s.s.*, *G. borealis* and *G. densiflora* (Bateman, Pridgeon & Chase 1997; Meekers *et al.* 2012). There are variations in flowering phenology between the three species and whilst relatively minor divergences occur between *G. conopsea* and *G. borealis* (Harrap & Harrap 2005), the peak flowering period of *G. densiflora* can be several weeks later (Meekers *et al.* 2012) . The herbarium specimens of *G. conopsea s.l.*, held at the Natural History Museum (BM) had not been taxonomically differentiated between the three species (formerly subspecies) and hence this collection was not used. Herbarium records held at Kew, however, had been taxonomically reviewed by R. Bateman: thus specimens of *G. conopsea s.s* were reliably identified and used in this analysis.

G. conopsea s.s is present throughout England, but is largely restricted to chalk grassland habitats (Fig. 3-6a) and limestone pastures; probably most abundant on the downlands of southern counties, and is rare in Scotland. Principal communities are

mesotrophic (MG3) and chalk (CG2-3, CG8) grasslands (Rodwell 1992). Maximum elevation in the British Isles has been recorded as 365 m (Pearman & Corner 2003).

A strong scent is produced on the surface of the floral parts and relative amounts of active compounds alter over a 24 hour cycle, with strongest scent emission at dusk (Huber *et al.* 2005). Nectar is freely produced and key pollinators are day and night-flying moths as well as butterflies (Meekers *et al.* 2012). Pollinator species include the Sphingidae (hawkmoths) *Macroglossum stellatarum* and *Deilephila porcellus* (van der Cingel 1995), and moths from the family Noctuidae, including *Autographa gamma* (Darwin 1877; Jersáková & Kindlmann 2004; Claessens & Kleynen 2011) and *A. bractea (Huber et al. 2005).* Other named pollinators include moths of the family Zygaenidae including *Zygaena filipendulae* (van der Pijl & Dodson 1966; van der Cingel 1995) and of the family Hesperiidae, such as *Ochlodes venata* (Huber *et al.* 2005; Claessens & Kleynen 2011). Occasional pollinators also include the bumblebee species *Bombus terrestris* and *B. pascuorum* (Claessens & Kleynen 2011) . Rates of fruit set are variable: whilst rates of 78-91% have been reported (Hansen & Olesen 1999), lower rates have been found in smaller populations (Meekers & Honnay 2011), suggesting that fruit set may be pollinator limited in small populations.

3.2.2.3.2 Platanthera chlorantha (Custer) Rchb. (syn. *Gymnadenia chlorantha* (Custer) Ambrosi, *Habenaria chlorantha* (Custer) Bab., *Orchis chlorantha* Custer) Greater Butterfly Orchid

P. chlorantha and *P. bifolia* are closely related species which frequently occur sympatrically (Nilsson 1983b). Recent molecular analysis indicates an exceptionally low level of genetic divergence between the species, with less than 0.7% sequence divergence in ITS analysis (Bateman *et al.* 2009). Whilst antheses of the two species overlap, *P. chlorantha* is the earlier to flower and the low levels of hybridisation reported are due to the interaction of ethological, morphological and temporal barriers (Nilsson 1983b). Following currently recognised taxonomy they are treated in this thesis as separate species.

P. chlorantha has an broad range across mainland Europe, extending northwards from Spain to Scandinavia and eastwards to Turkey, and has been recorded at altitudes of c. 2,000 m in the Alps (Foley & Clarke 2005).

In Britain *P. chlorantha* is in essence a lowland species, but has been recorded at *c.* 455 m in Northumberland and at *c.* 335 m in Scotland (Wilson 1956). Primarily associated with base-rich or calcareous soils, but tolerant of mild acidity (Harrap & Harrap 2005).. Its principal NVC classification is within the *Fraxinus excelsior-Acer campestre-Mercurialis perennis* woodland (W8) (Rodwell 1991a). It is fairly widely distributed across southern, central and north west England, Northern Ireland, and Wales but less frequently reported across western Scotland, and is declining in range. Flowering period is early May to late July.

Scent from the flowers is strongest at night, and copious quantities of nectar within the spur serve to attract pollinators (Nilsson 1978), chiefly Noctuid (Proctor & Yeo 1973; van der Cingel 1995) and Sphingid moths (Claessens & Kleynen 2011). Sphingid moths include the Elephant Hawk moth (*Deilephila elpenor*), the Small Elephant Hawk moth (*Deilephila porcellus*) and the Silver Y moth (*Autographa gamma*) (Nilsson 1978; Claessens & Kleynen 2011).

3.2.2.3.3 Platanthera bifolia (L.). Rich. (syn. *Gymnadenia bifolia* (L.) G Mey, *Habenaria bifolia* (L.) R. Br., *Orchis bifolia* L.) Lesser Butterfly Orchid

The species is widely distributed in Europe, and its north-south range extends from the Mediterranean and North Africa to Scandinavia, and eastwards to Russia.

Although present throughout the British Isles, it is most frequently recorded in the western regions of England and Wales and the north-west of Scotland (Foley & Clarke 2005). Essentially a lowland plant in Britain, (Wilson 1956), it is present on both basic and acidic soils. In southern England it favours woodland sites, typically over basic soils, but also occupies open, damp acidic habitats. In the north and west it favours open, acidic, damp conditions such as open heath, moorland and pasture (Foley & Clarke 2005). Morphological variation is reported in Britain: woodland forms being slightly taller than those of open acidic habitats, with narrower leaves and a looser inflorescence (Foley & Clarke 2005).

The main distinguishing feature between the two species is the orientation and shape of the pollinia, which in *P. bifolia* are positioned close together and vertically parallel (Foley & Clarke 2005). Range in Britain has contracted in many central and Southern regions over the last few decades, probably due to habitat loss, and current distribution has a distinct western and northern bias (Harrap, 2005). The species is classified as Vulnerable in the current Red Data List of Vascular Plants for Great Britain (Cheffings *et al.* 2005). Flowering period is described as late May to June for woodland populations and June to July for heathland populations, with southerly populations flowering earliest (Harrap, 2005).

In common with *P. chlorantha*, the species emits a strong scent. especially at night, and offer abundant nectar within the floral spur (Plepys, Ibarra & Löfstedt 2002). Primary pollinators are night-flying sphingid and noctuid moths (van der Cingel 1995), also geometrid moths (Plepys, Ibarra & Löfstedt 2002). Named pollinator species include *Deilephila elpenor, D. porcellus, Macroglossum stellatarum, Cucullia umbratica*, and *Autographa gamma* (Plepys, Ibarra & Löfstedt 2002; Jersáková & Kindlmann 2004; Claessens & Kleynen 2011).

3.2.2.3.4 Epipactis purpurata Sm. (syn. *Helleborine purpurata* (Sm.) Druce, *Helleborine sessilifolia* (Peterm.) Druce) Violet Helleborine

Epipactis purpurata is restricted to western and central Europe, extending north from the western Iberian Peninsula, across central Europe to Denmark and eastwards to the Balkans and toward Siberia (Foley & Clarke 2005). Within Britain it is a lowland plant (Wilson 1956) restricted to the southeast and central England, where it is strongly associated with shady woodland habitats, over calcareous substrate (Foley & Clarke 2005; Harrap & Harrap 2005). Within NVC classification, it is primarily allied with *Fagus sylvatica-Rubus fruticosus* woodland (W14) (Rodwell 1991a). Flowering occurs between late June and late September (Harrap & Harrap 2005).

The primary pollinators of *Epipactis* species in Europe are reported to be vespid wasps (Hymenoptera: Vespidae) (Jakubska-Busse & Kadej 2011). Little is known of the specific pollinators for *E. purpurata*: pollinating wasps include *Vespula austriaca* (Claessens & Kleynen 2011) and *V. vulgaris*, (Jakubska-Busse & Kadej 2011) and short-headed species of the genus *Paravespula* (van der Cingel 1995; Claessens &

Kleynen 2011). In a central European study pollinators were scarce and plants exhibited high levels of autogamy (Jakubska-Busse & Kadej 2011).

3.2.2.3.5 Spiranthes spiralis (L.) Chevall (syn. *Spiranthes autumnalis* (Balb.) Rich.) Autumn Lady's-tresses

Spiranthes spiralis is an autumn flowering species, whose flowering period spans August and September, into early October. Although widely distributed across southern England, occurrence is sporadic further north, reaching its current recorded geographic limit in Lancashire (Lang 2004). It is also found in Wales where it occurs along coastal fringes, as it does in mid to southern Ireland. Habitat is essentially short calcareous grassland, where competition from taller grasses and herbs is limited (Harrap & Harrap 2005). It is principally found amongst the CG2 (*Festuca ovina-Avenula pratensis*) grasslands (Rodwell 1992) but also occurs within CG10 (*Festuca ovina-Agrostis capillaris-Thymus polytrichus*) communities (Jacquemyn & Hutchings 2010). In Wales it has also been recorded in *Cynosurus cristatus-Centaurea nigra* (MG5) mestrotrophic grasslands (Jacquemyn & Hutchings 2010).

S. spiralis is long lived, and total life span above ground has been estimated as *c.* 60 years (Wells 1981; Willems & Dorland 2000). During mid-summer plants remains underground; peak flowering period is August to September, although plants may still be flowering as late as November (Willems & Dorland 2000).

Hymenoptera are primary pollen vectors (Darwin 1877; van der Cingel 1995; Willems & Lahtinen 1997; Jacquemyn & Hutchings 2010). Named pollinators include *Bombus pascuorum, B. lapidarius*, *B. terrestris* and *Apis mellifera* (Willems & Lahtinen 1997; Claessens & Kleynen 2011).

3.2.2.3.6 Neottia ovata (L.) R.Br. (syn. *Listera ovata* (L.) R.Br., *Ophrys ovata* L.) Common Twayblade

Neottia ovata is widespread and common through much of Europe, and its range extends northwards to Scandinavia and eastwards to central Asia (Foley & Clarke 2005). In the British Isles it is also widespread; habitats include calcareous (Fig. 3-6b) and mildly acidic grasslands, woodlands, heathland and dune slacks. Principal NVC classifications are the *Anthoxanthum odoratum-Geranium sylvaticum* (MG3) and

Cynosurus cristatus-Centaurea nigra (MG5) mesotrophic grasslands, and chalk grassland communitiues (CG6-13) (Rodwell 1992).

A shallow nectariferous groove runs centrally down the lip from the base, attracting a wide range of insects (Lang 1989; Delforge 1995). Flowering occurs from late April through to July. *N. ovata* is rhizomatous, and vegetative growth is an important means of reproduction, with seed to plant maturity reportedly up to 15 years (Lang 2004). Effective pollinators are small insects (Nilsson 1981), including Hymenoptera, Coeloptera and Diptera (Darwin 1877; Nilsson 1981; Claessens & Kleynen 2011).

Table 3- 2. Summary of study species.

Species Name	Median Flowering Date	Pollination Mode	Pollinator Assemblage	Primary NVC Groups	Primary Geographic Regions
Ophrys sphegodes	17 May	Sexual deceit	Hymenoptera	CG	South (S)
Ophrys insectifera	8 June	Sexual deceit	Hymenoptera	CG; W12; M13	S
Orchis mascula	13 May	Food deceit	Hymenoptera	CG13; MG3; U17	Widespread, but localised
Anacamptis pyramidalis	6 July	Food deceit	Lepidoptera	CG1-6; MG1; MG5	S; Central (C); East (E)
Anacamptis morio	17 May	Food deceit	Hymenoptera	MG5; CG	S; Central (C); East (E)
Dactylorhiza fuchsii	25 June	Food deceit	Generalist	MG3; MG9; CG 2-3	Widespread
Dactylorhiza praetermissa	17 June	Food deceit	Generalist	M9-13; M22-24; S24; SD	S; SE
Cephalanthera longifolia	5 June	Food deceit	Hymenoptera	W12	S
Neotinea ustulata	6 June	Food deceit	Generalist	CG2	S
Gymnadenia conopsea s.s.	24 June	Reward	Lepidoptera	CG2-3; CG8; MG3	Widespread
Platanthera chlorantha	13 June	Reward	Lepidoptera	W8	Widespread, but localised
Platanthera bifolia	17 June	Reward	Lepidoptera	W8	Widespread, but localised
Epipactis purpurata	18 August	Reward	Hymenoptera	W14	S; C
Spiranthes spiralis	5 September	Reward	Hymenoptera	CG2; CG10	S; C
Neottia ovata	13 June	Reward	Generalist	MG3; MG5; CG6-13	Widespread

3.2.3 Meteorological data

Mean monthly Central England Temperature (CET) records and mean monthly precipitation figures for England and Wales (EWP) for the period 1820-2010 (Parker *et al*. 1992) were obtained from the UK Meteorological Office as described in Chapter 2, section *2.2.4*.

Three-month mean temperature data was calculated for each year (Winter-December (the prior year), January, February; Spring-March, April, May; Summer-June, July, August; Autumn of the prior year-September, October, November).

Mean precipitation figures calculated for each year (Winter-December (the prior year), January, February; Spring-March, April, May; Autumn of the prior year-September, October, November).

3.2.4 Geographical effects

A vice-county is a geographical division of the British Isles used for the purposes of biological recording. The vice county of collection is often recorded on a herbarium specimen, even when the precise location of collection is not given. For many historical specimens accurate details of location beyond vice county are not provided. Therefore the effect of latitude and longitude on flowering date was based on coordinates of vice county centroids. Decimal latitude and longitude (WSG86 coordinates) of vice county centroids were calculated from their XY values. The collection site of each specimen was geo-referenced with the aid of the Ordnance Survey UK Grid Reference Finder http://gridreferencefinder.com/ and assigned to its vice county using the Digitised Watsonian Vice County Boundary data accessed via Herbaria United and the Botanical Society of the British Isles http://herbariaunited.org/gridrefVC/

3.2.5 Analysis

The distribution of collection dates in the herbarium datasets for each of the 15 species were expressed as number of days after 1st March. Each dataset was checked for normality and presence of outliers. Median and range of first and last flowering date were calculated for each of the study species. All sets of flowering phenology data were examined for correlations (Pearson Correlation Coefficient) with mean CET

temperature data from the months prior to the flowering season. These data included mean monthly temperature and its averages over successive 3-month periods (September-November, December-February and March-May). All sets of flowering phenology data were also examined for correlations with mean EWP precipitation data from the months prior to the flowering season. These data included mean monthly precipitation and its averages over successive 3-month periods (September-November, December-February and March-May). Multiple regression analyses were used to investigate models of the dependence of flowering time on mean temperatures, precipitation, decimal latitude and decimal longitude, in order to identify sensitivity of these models to monthly temperature and precipitation means and varying latitude and longitude.

Stepwise forward multiple regression analyses were carried out using SPSS 18 (IBM Corp., 1 New Orchard Road, Armonk, NY, USA). Slopes and intercepts of regressions were calculated and compared via analysis of covariance (ANCOVA) using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA (www.graphpad.com).

3.3 Results

3.3.1 Quality of herbarium data

Of the 4,046 herbarium specimens examined 2,176 (53.8%) provided geo-referenceable, dated records of plants in peak flower at the time of collection (i.e. more than 40% open flower, and neither in seed nor with senescing flowers). Of these specimens, 1,803 were collected from English vice counties, and thus within the range of the CET records (Table 3-3). Considerable variation between species was evident in the proportion of usable records, which was principally due to wastage arising from undated specimens.

3.3.2 Median and range of flowering times

Of the five species pollinated by Hymenoptera (Table 3-4) two are late season (*Epipactis purpurata* and *Spiranthes spiralis*) and three are early season (*Orchis mascula*, *Anacamptis morio* and *Cephalanthera longifolia*). For *O. mascula* median flowering (days from 1st March) was 74 days and range was 45 to 124 days (Fig. 3-7).

For *A. morio* median flowering (days from 1st March) was 78, whilst flowering range extended from 47 to 110 days (Fig. 3-7). *C. longifolia* is an orchid of early summer, median flowering (days from 1st March) was 97 days and flowering range was between 67 and 147 days (Fig. 3-7). For *E. purpurata* median flowering time (days from 1 March) was 171 days and flowering range was between 129 and 212 days (Fig. 3-7). For *S. spiralis* median flowering (days from 1st March) was 189 days and flowering range was 162 to 223 days (Fig. 3-7).

Ophrys sphegodes and *O. insectifera* were the only species in this study to rely on sexual deceit for pollination success (Table 3-7). *O. sphegodes* is very early to flower with a median flowering date (days from 1st March) of 78 days and a range of 47 to 112 days (Fig. 3-7). For *O. insectifera*, flowering in early summer, median flowering date (days from 1st March) was 100 days and range was 76 to 132 days (Fig. 3-7).

Of the four species pollinated by Lepidoptera (Table 3-5) *Platanthera bifolia* and *P. chlorantha* were the earliest to flower. Median flowering date (days from 1st March) for *P. bifolia* was 109 days and flowering range was 84-139 days (Fig. 3-7). For the marginally earlier flowering *P. chlorantha* median flowering date was 105 days and range was 75-139 days (Fig. 3-7). For *G. conopsea* median flowering (days from 1st March) was 116 days. The flowering range, spanning 94 days (range 86-179 days), was the most extended of the study species (Fig. 3-7). *Anacamptis pyramidalis* was the last to flower of the Lepidopteran pollinated orchids in this study. Median flowering date (days from 1st March) was 128 days and ranged from 98 to 170 days (Fig. 3-7).

Of the pollinator generalists (Table 3-6) *Neottia ovata* was the sole rewarding species studied. Median flowering date (days from 1st March) was 105 days and range extended from 79 to 157 days (Fig. 3-7). Of the three non-rewarding species of pollinator generalists in this study *Neotinea ustulata* was the earliest to flower: median flowering date (days from 1st March) was 98 days and ranged from 62 to 124 days (Fig. 3-7): presumably all specimens were of the more common early flowering var. *ustulata*. For *Dactylorhiza praetermissa* flowering was marginally later, with a median flowering date (days from 1st March) of 109 days and a range of between 76 and 143 days (Fig. 3-7). *Dactylorhiza fuchsii* was the last to flower, median flowering date (days from 1st March) was 117 days and flowering range 93 to 157 days (Fig. 3-7).

Table 3-3. Summary of records derived from herbarium specimens.

Orchid species	Total	Undated	Scotland, Wales, Isle of Man	< 40% open flower	Senesced flower or in seed	Usable records
			Number of herbarium specimens			
Anacamptis morio	289	147	7	1	8	126
Anacamptis pyramidalis	330	159	17	-	3	151
Cephalanthera longifolia	160	74	23	6	3	54
Dactylorhiza fuchsii	315	44	26	47	12	186
Dactylorhiza praetermissa	387	42	40	20	24	261
Epipactis purpurata	212	76	-	23	9	104
Gymnadenia conopsea	192	46	50	7	-	89
Neotinea ustulata	234	136	-	1	5	92
Neottia ovata	180	51	28	14	1	86
Ophrys insectifera	285	155	-	9	7	114
Ophrys sphegodes	183	102	-	1	3	77
Orchis mascula	364	170	30	9	11	144
Platanthera bifolia	363	159	85	5	4	110
Platanthera chlorantha	286	120	53	2	2	109
Spiranthes spiralis	266	141	14	6	6	100
Total records	4046	1622	373	151	97	1803
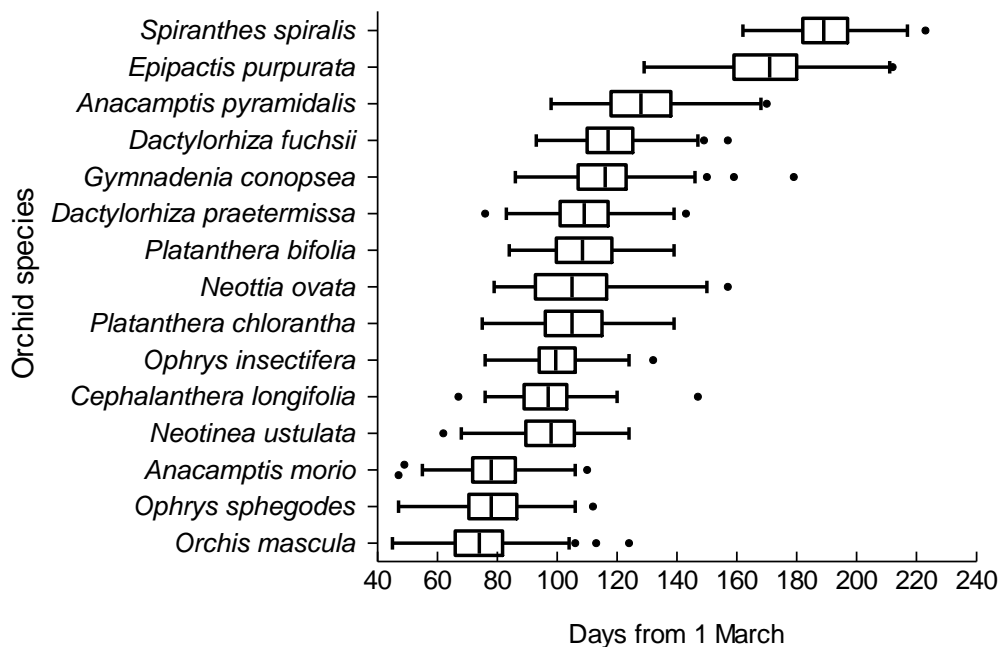
Figure 3-7. Median flowering dates (days from 1 March), 25 and 75 percentiles, range and outliers for each of the 15 orchid species derived from herbarium records.

3.3.3 Correlation analysis

3.3.3.1 Mean CET

Species were analyzed according to their mode of pollination and flowering period. All three species of Hymenoptera-pollinated early-season orchids demonstrated strong correlation with mean spring temperature. Flowering of *Orchis mascula* was significantly correlated with mean monthly temperature for the months January-April. Whilst there was significant correlation with mean winter temperature the highest correlation was with spring (Table 3-4). Flowering of *Anacamptis morio* was significantly correlated with monthly mean temperatures for the months February-April. Significant correlation was present with mean winter temperature but strongest correlation was with spring (Table 3-4). For *Cephalanthera longifolia* there were significant correlations between flowering date and mean monthly temperature for the months February-May, and for July. There was significant correlation with mean temperature for winter but the highest correlation was with mean temperature for spring (Table 3-4). For the two late-season orchids in this Hymenoptera-pollinated group, peak flowering showed significant correlation with monthly temperature through the growing season. Time of flowering of *Epipactis purpurata* was significantly correlated with mean monthly temperatures for the 6 months prior to flowering (March-August). As expected there was strong correlation with spring but the highest correlation was with summer immediately preceding flowering (Table 3-4). Flowering of *Spiranthes spiralis* was significantly correlated with the mean monthly temperatures for January, March, May and June. There were significant correlations with mean temperatures for winter and summer but the strongest correlation was with spring (Table 3-4).

Turning to the orchids pollinated by Lepidoptera, the *Platanthera spp*., both of which offer nectar reward, were the earliest to flower. For *P. chlorantha* there were significant correlations with mean monthly temperatures for February, March, and April. Again the strongest correlation was with mean temperature for spring (Table 3-5). For *P. bifolia* there were significant correlations with mean monthly temperatures for March, April and June and with mean winter temperature, however the strongest correlation was also with mean spring temperature (Table 3-5). The two non-rewarding

species in this group commenced flowering later than the *Platanthera spp.* and flowered for longer thorough the summer. The wide range in flowering time of *Gymnadenia conopsea s.s.* made it difficult to apply correlation analysis to flowering date and therefore correlation analysis was restricted to the 75 specimens collected from the southern/central region of England; 14 specimens originating from northern vice counties were excluded in this analysis. Despite this geographic concentration of records, correlation analysis yielded weak results. Flowering date was significantly correlated with mean monthly temperature for February and May and with mean winter temperature (Table 3-5). For *Anacamptis pyramidalis* there were significant correlations with mean monthly temperatures between February and August and with mean temperatures for winter and spring, however the strongest correlation was with mean summer temperature (Table 3-5).

Correlation analyses for the rewarding pollinator generalist *Neottia ovata* produced weak correlations: flowering date was significantly correlated only with mean monthly temperature for April (Table 3-6). Turning to the 3 species of non-rewarding pollinator generalists, there were significant correlations for *Neotinea ustulata* with mean monthly temperatures for February, April and May and with mean spring temperature (Table 3-6). For *Dactylorhiza praetermissa*, flowering marginally later than *N. ustulata*, there were significant correlations with mean monthly temperatures in the period February-June and with mean summer temperature. The highest correlation was with spring (Table 3-6). For *Dactylorhiza fuchsii* there were significant correlations with mean monthly temperature for February and for the months April – July, and significant correlation with mean spring temperature. Highest correlation however, was with mean summer temperature (Table 3-6).

Ophrys sphegodes and *O. insectifera* were the only species in this study to rely on sexual deceit for pollination. Of these, *O. sphegodes* was the earliest to flower, and significant correlations were observed with mean monthly temperature for March and May. Whilst there was significant correlation with mean winter temperature, the strongest correlation was again with spring (Table 3-7). For *O. insectifera* there were significant correlations with mean monthly temperature for February to April, and also June and July. Significant correlations were observed with the mean temperature for

autumn of the prior year (September- November), and with mean spring temperature, however the highest correlation was with mean winter temperature (Table 3-7).

Table 3-4. Hymenoptera pollinated orchids. Comparison of correlations between flowering date, temperature, precipitation latitude and longitude for herbarium records. Correlations are shown with mean temperature or precipitation for 3-monthly periods and individual months in the same year as flowering (January-August) or in the year previous to flowering (September-December). A negative correlation indicates that a higher mean temperature or precipitation is associated with an earlier flowering date.

	Rewarding		Non-rewarding		
	Epipactis purpurata (1844-1977) *n = 104*	*Spiranthes spiralis* (1823-1973) *n = 100*	*Orchis mascula* (1835-1980) *n = 144*	*Anacamptis morio* (1835-1981) *n =126*	*Cephalanthera longifolia* (1848-1957) *n = 54*
Period of temperature mean:					
July	[2] -0.451**	-0.119	[3] 0.061	-0.005	-0.300**
June	[1] -0.526**	[2] -0.307**	-0.050	-0.137	-0.092
May	-0.345**	[1] -0.329**	-0.103	-0.124	[2] -0.325**
April	[3] -0.246**	-0.153	[1] -0.325**	[1] -0.349**	[1] -0.403**
March	- 0.166*	-0.203*	-0.266**	[2] -0.305**	[3] -0.342**
February	- 0.059	-0.120	[5] -0.307**	-0.234**	-0.309*
January	0.041	[3] -0.207*	[2] -0.200**	-0.030	-0.168
3 month means:					
June - August	-0.553**	-0.243**	-	-	-
March - May	-0.398**	-0.353**	-0.364**	-0.406**	-0.548**
December [Y]– February	-0.002	-0.323**	-0.262**	-0.173*	-0.351**
September – Nov. [Y]	0.064	-0.032	0.118	-0.058	-0.119
Period of precipitation 3 month means:					
March - May	-0.082	0.086	0.118	0.051	-0.068
December [Y] -February	0.108	-0.167*	-0.124	0.047	-0.093
September – Nov. [Y]	-0.217*	0.074	-0.007	0.175*	0.243*
Decimal latitude	-0.074	-0.016	[4] 0.189*	0.087	0.093
Decimal longitude	-0.102	0.004	-0.139*	-0.070	0.007

Significance: **P <0.01; * P <0.05 (one-tailed) **Symbols:** [Y] = prior year; [1] [2] [3] [4] [5] = order in stepwise (forward) regression

Table 3-5. Lepidoptera pollinated orchids. Comparison of correlations between flowering date, temperature, precipitation, latitude and longitude for herbarium records. Correlations are shown with means for 3-monthly periods and individual months in the same year as flowering (January-August) or in the year previous to flowering (September-December). A negative correlation indicates that a higher mean temperature or precipitation is associated with an earlier flowering date.

	Rewarding			Non rewarding
	Platanthera chlorantha (1835-1962)	*Platanthera bifolia* (1852-1965)	*Gymnadenia conopsea* [a,K] (1843-1986)	*Anacamptis pyramidalis* (1830-1980)
	n = 109	*n* = 110	*n* = 75 [a]	*n* = 151
Period of temperature mean:				
July	-0.122	-0.127	0.068	-0.280**
June	-0.090	0.173*	-0.026	[1] -0.344**
May	[3] -0.103	-0.134	[3] -0.232*	-0.185*
April	-0.258**	[4] -0.233**	0.083	[2] -0.343**
March	-0.203*	[2] -0.384**	-0.001	-0.239**
February	[4] -0.194*	-0.147	[2] -0.284**	-0.238**
January	0.049	-0.101	-0.180	-0.104
3 month means:				
June - August	-	-	-	-0.414**
March - May	-0.289**	-0.374**	-0.070	-0.387**
December [Y] -February	-0.132	-0.222**	-0.257*	-0.279**
September – November [Y]	0.110	0.011	0.082	-0.096
Period of precipitation 3 month means:				
March - May	0.149	0.041	0.182	-0.008
December [Y] -February	0.051	-0.090	-0.186	-0.218**
September – November [Y]	-0.086	-0.155	-0.153	-0.060
Decimal latitude	[1] 0.447**	[1] 0.422**	[1] 0.431**	0.198**
Decimal longitude	[2] -0.297**	[3] -0.273**	0.194*	0.121

Significance: **P <0.01; * P <0.05 (one-tailed), **Symbols:** [a] = region 1 only; [K] Kew specimens only; [Y] = prior year; [1] [2] [3] [4] = order in stepwise (forward) regression

Chapter 3

Table 3-6. Pollinator generalists. Comparison of correlations between flowering date, temperature, precipitation, latitude and longitude for herbarium records. Correlations are shown with means for 3-monthly periods and individual months in the same year as flowering (January-August) or in the year previous to flowering (September-December). A negative correlation indicates that a higher mean temperature or precipitation is associated with an earlier flowering date.

	Rewarding		Non-rewarding	
	Neottia ovata [K] (1829-1979) n = 86	*Neotinea ustulata* (1823-1955) n = 92	*Dactylorhiza praetermissa* [K] (1870-1972) n = 261	*Dactylorhiza fuchsii* [K] (1850-1973) n = 186
Period of temperature mean:				
July	0.153	-0.063	-0.001	-0.198**
June	-0.062	-0.079	-0.160**	-0.175**
May	-0.072	[2] -0.215*	[1] -0.214**	[2] -0.169*
April	[2] -0.256**	-0.240*	-0.144**	-0.175**
March	0.023	-0.168	-0.155**	-0.030
February	-0.052	-0.253**	[3] -0.119*	-0.128*
January	0.085	0.156	0.031	-0.041
3 month means:				
June- August	0.062	-0.109	-0.140*	-0.285**
March - May	-0.127	-0.298**	-0.263**	-0.168*
December [Y] -February	-0.046	-0.104	-0.102	-0.081
September – November [Y]	0.018	0.041	0.071	0.029
Period of precipitation 3 month means:				
March - May	0.063	-0.142	0.045	-0.038
December [Y] -February	0.120	-0.025	0.069	-0.031
September – November [Y]	0.038	-0.133	-0.041	0.005
Decimal latitude	[1] 0.504**	0.003	0.095	[1] 0.407**
Decimal longitude	0.192*	[1] -0.285**	[2] 0.214**	-0.025

Significance: **P <0.01; * P <0.05 (one-tailed), **Symbols:** [K] = Kew specimens only, [Y] = prior year; [1] [2] [3] = order in stepwise (forward) regression

3.3.3.2 Mean England and Wales Precipitation

For the majority of species correlations of flowering date with mean monthly precipitation were not significant.

For *Epipactis purpurata* there were significant positive correlations with mean monthly precipitation for May to July, though none were significant in stepwise forward regression, possibly due to co-linearity with monthly temperature variables.

There were also significant positive correlations with mean monthly precipitation for January for *Ophrys insectifera* and *Dactylorhiza praetermissa*, with mean monthly precipitation for March for *Platanthera chlorantha*, April for *Neottia ovata*, May for *Anacamptis morio*, and June for *Spiranthes spiralis* and *D. fuchsii* although none were significant variables in multiple stepwise regression analysis.

There were significant negative correlations with mean monthly precipitation for January for *P. bifolia*, with February for *O. insectifera* and for *Anacamptis pyramidalis*, with March for *E. purpurata*, and with April for *Cephalanthera longifolia*, although none were significant in multiple stepwise regression analysis.

Table 3-7. Orchids pollinated by sexual deceit: Comparison of correlations between flowering date and temperature, precipitation, latitude and longitude for herbarium records. Correlations are shown with means for 3-monthly periods and individual months in the same year as flowering (January-July) or in the year previous to flowering (September-December). A negative correlation indicates that a higher mean temperature or precipitation is associated with an earlier flowering date.

	Sexual Deceit	
	Ophrys sphegodes (1848-1958) *n = 77*	*Ophrys insectifera* (1835 – 1970) *n = 114*
Period of temperature mean:		
July	-	[1] -0.338**
June	-0.091	[3] -0.225**
May	-0.259*	-0.108
April	-0.153	[4] -0.280**
March	[2] -0.396**	-0.255**
February	-0.159	[2] -0.307**
January	-0.003	0.056
3 month means:		
March - May	-0.426**	-0.330**
December [Y]– February	-0.321**	-0.228**
September – Nov. [Y]	-0.065	0.084
Period of precipitation 3 month means:		
March - May	0.158	-0.015
December [Y] -February	0.115	-0.024
September – Nov. [Y]	0.042	-0.011
Decimal latitude	0.373**	-0.120
Decimal longitude	[1] 0.427**	-0.039

Significance: **P <0.01; * *P* <0.05 (one-tailed), **Symbols:** [Y] = prior year; [1] [2] [3] [4] = order in stepwise (forward) regression

3.3.3.3 Geographic variation

For most species the effect of latitude and longitude was difficult to assess because northern sites were generally more westerly due to the geography of the British Isles. For rare species the effect of latitude and longitude was confounded by repeated collection at a limited number of sites. Several species registered significant

correlations with latitude and longitude as well as mean spring, or summer, temperature. For these species (*A. pyramidalis*, *D. fuchsii*, *D. praetermissa*, *N. ustulata*, *O. mascula*, *O. sphegodes*, *P. bifolia* and *P. chlorantha*) partial correlation analysis of flowering data and mean spring temperature, after controlling for latitude and longitude effects, confirmed the significance of mean spring, or summer, temperature for flowering date in each of the orchids.

Table 3-8. Zero-order correlations of flowering date (days from March) on mean spring temperature (MST) and partial correlations controlling for latitude and longitude.

	Zero-order correlation of flowering date with Mean Spring Temperature (MST).	Partial correlation of flowering date with MST, controlling for latitude and longitude.
A. pyramidalis (n=151)	-0.387**	-0.387**
D. fuchsii (n=186)	-0.168*	-0.173**
D. praetermissa (n=261)	-0.263**	-0.234**
N. ustulata (n=92)	-0.298**	-0.365**
O. mascula (n=144)	-0.364**	-0.345**
O. sphegodes (n=77)	-0.426**	-0.326**
P. bifolia (n=110)	-0.374**	-0.386**
P. chlorantha (n=109)	-0.289**	-0.305**

Significance: ** $P < 0.01$; *$P < 0.05$

3.3.4 Multiple regression analysis

For each orchid species stepwise forward regression analysis was used to identify individual months that were of high significance. As expected mean monthly temperature in the months immediately prior to flowering were consistently identified as key variables. Stepwise forward regression did not produce a common model suitable for orchid species grouped either by pollinator type or by flowering period. Mean monthly temperature in March, April or May was identified as a key variable for every orchid species (Table 3-4, Table 3-5, Table 3-6, Table 3-7).

For 13 of the 15 orchid species in this study mean spring (March-May) temperature was significantly correlated with flowering time indicating the importance of spring temperature in relation to flowering phenology. The two exceptions were *G. conopsea* and *Neottia ovata*. Both species showed generally weak responses in correlation

analyses, which was not unexpected due to their long flowering seasons, coupled with wide geographic and habitat range.

For each of the three early flowering Hymenoptera pollinated species mean monthly temperature for March (*Ophrys sphegodes*) (Table 3-7) and April (*Orchis mascula* and *Anacamptis morio*) (Table 3-4) was a key factor. *Cephalanthera longifolia* flowering marginally later followed a similar pattern; mean monthly temperature for March-May were prime factors (Table 3-4).

For the two late-summer flowering species of Hymenoptera-pollinated orchids, summer temperatures as well as those of spring were important in stepwise forward regression. For *Epipactis purpurata* mean monthly temperatures in April, June and July were key factors in stepwise forward regression. For *Spiranthes spiralis*, flowering at its peak during September, mean monthly temperature in January, May and June were identified in stepwise forward regression (Table 3-4).

Of the sexually deceptive orchids *Ophrys sphegodes* flowered earlier, and March temperature was flagged as a key factor. *For Ophrys insectifera* key variables in stepwise forward regression were mean temperatures for February, April, June and July (Table 3-7).

Mean temperatures for February and May were both significant in two of the four species pollinated by Lepidoptera; *Platanthera chlorantha* and *Gymnadenia conopsea* (Table 3-5), despite weak overall correlation and regression results for *G. conopsea.* For *P. bifolia* spring temperature was again important, mean monthly temperatures for March and April were key variables. Interestingly, for each of these three species, latitude of vice county of collection was identified as a key variable in stepwise forward regression (Table 3-5). For *A. pyramidalis* mean temperature for June was the prime temperature variable in stepwise forward regression. Latitude of vice county of collection was also significant, reflecting the recognized gradient of later flowering with northing.

Of the pollinator generalists the non-rewarding species *Dactylorhiza praetermissa* and, *Neotinea ustulata*, showed similar patterns in stepwise forward regression: May temperature was a key factor for both species whilst mean temperature for February was also important for *D. praetermissa* (Table 3-6). For *Neotinea ustulata* (Table 3-6) negative association with longitude was also flagged, suggesting earlier flowering with easting. This association may be a result of the co-linearity between latitude and longitude (-0.305, $p < 0.001$) since collection sites in the west tended also to be more northerly. Analysis of vice county of collection for *N. ustulata* revealed a distinct binomial distribution: a large set of southern vice counties and a smaller group of northern collection sites.

3.3.5 Linear regression analysis

For nine of the orchid species mean spring (March-May) temperature produced the highest correlation with flowering time, and was identified as the strongest basis for comparing flowering response to temperature in linear regression. The relationship between flowering and mean spring temperature was established for each of the study species (Table 3-9).

For two non nectar-rewarding orchid species pollinated by Hymenoptera, and earliest to flower, regression of flowering date on mean spring temperature yielded markedly similar results. For *Orchis mascula* the regression of flowering date on mean spring temperature accounted for 13.2% of the variation in flowering time (Fig. 3-8a). A 1°C increase in mean spring temperature was associated with an advance in flowering of 5.96 days. For *A. morio* linear regression of flowering date on mean spring temperature accounted for 16.5% of the variation in flowering time (Fig. 3-8b) and a 1°C increase in mean spring temperature was associated with an advance in flowering of 6.02 days. The slopes of the regression models for the two species were statistically indistinguishable ($F_{1,266} = 0.0013$, $P = 0.972$) demonstrating a common flowering response to temperature. Even so, the intercepts were statistically different ($F_{1,267} = 5.0061$, $P = 0.027$).

For the sexually deceptive orchid *Ophrys sphegodes* linear regression on flowering date accounted for 18.2% of the variation in flowering time (Fig. 3-9), and a 1°C

increase in mean spring temperature was associated with an advance in flowering of 6.5 days. The regression models for *O. sphegodes* and the non nectar-rewarding *Orchis mascula* were statistically indistinguishable, neither the gradients ($F_{1,217} = 0.0663$, $P = 0.797$) nor the intercepts ($F_{1,218} = 0.2278$, $P = 0.634$) were significantly different. Similarly the regression models for *Ophrys sphegodes* and the non nectar-rewarding *A. morio* were statistically indistinguishable, again neither the gradients ($F_{1,199} = 0.0576$, $P = 0.811$) nor the intercepts ($F_{1,200} = 2.1142$, $P = 0.148$) were significantly different.

Table 3-9. Relationship between flowering date (days after 1 March) and mean spring (March-May) temperature for the 15 orchid species in the study.

Species Name	*y* constant	*S.E* +/-	*x* slope	*S.E* +/-	r^2	*n*
Hymenoptera pollinated:						
Orchis mascula (i)	125.2	10.91	-5.96**	1.28	0.13	144
Anacamptis morio (i)	129.0	10.25	-6.03**	1.22	0.16	126
Cephalanthera longifolia (i)	166.3	14.68	-8.57**	1.81	0.30	54
Epipactis purpurata (ii)	231.3	14.11	-7.45**	1.70	0.16	104
Spiranthes spiralis (ii)	233.5	11.86	-5.36**	1.44	0.12	100
Sexually deceptive:						
Ophrys sphegodes (i)	130.5	13.04	-6.51**	1.60	0.18	77
Ophrys insectifera (i)	136.3	9.87	-4.43**	1.20	0.11	114
Lepidoptera pollinated:						
Platanthera chlorantha (ii)	145.1	12.51	-4.69**	1.50	0.08	109
Platanthera bifolia (ii)	152.6	10.44	-5.35**	1.28	0.14	110
Gymnadenia conopsea (ii)	124.8	17.08	-1.23	2.04	0.01	75
Anacamptis pyramidalis (i)	188.3	11.52	-7.10**	1.39	0.15	151
Pollinator Generalists:						
Neottia ovata (ii)	131.5	20.15	-2.79	2.38	0.02	86
Neotinea ustulata (i)	129.4	11.12	-4.05**	1.37	0.09	92
Dactylorhiza praetermissa (i)	145.0	8.11	-4.21**	0.96	0.07	261
Dactylorhiza fuchsii (i)	136.4	8.10	-2.21*	0.96	0.03	186

Significance: ** $P < 0.01$; * $P < 0.05$, **Symbols**: (i) non-rewarding, (ii) rewarding

Figure 3-8. Relationship between flowering date (days after 1 March) and mean spring (March – May) temperature for a) *Orchis mascula* ($y = 125.2 - 5.962x$, $r^2 = 0.132$, P < 0.001, n = 144) and b) *Anacamptis morio* ($y = 129.0 - 6.026x$, $r^2 = 0.165$, P < 0.001, n = 126.

For the sexually deceptive *O. insectifera* linear regression on flowering date accounted for 10.9% of the variation in flowering time (Fig. 3-9), and a 1°C increase in mean spring temperature was associated with an advance in flowering of 4.4 days.

Comparison of the two sexually deceptive orchids, *O. sphegodes* and *O. insectifera,* gave similar results. The slopes of the regression models for the two species were statistically indistinguishable ($F_{1,187} = 1.1211$, $P = 0.291$) (Fig. 3-9). As anticipated, the intercepts were dissimilar ($F_{1,188} = 195.876$, $P < 0.001$), reflecting the differentiation in time of onset of flowering.

Figure 3-9. Relationship between flowering date (days after 1 March) and mean spring (March – May) temperature for *Ophrys insectifera* ($y = 136.3 - 4.43x$, $r^2 = 0.109$, $P < 0.001$, $n = 114$) and *O. sphegodes* ($y = 99.54 - 6.51x$, $r^2 = 0.182$, $P < 0.001$, $n = 77$). Symbols: ● = data for *O. insectifera*; ● = data for *O. sphegodes.*

Linear regression of flowering date of the non nectar-rewarding *Cephalanthera longifolia* on mean spring temperature accounted for 30% of the variation in flowering time (Fig. 3-11) and a 1°C increase in mean spring temperature was associated with an advance in flowering of 8.6 days. Although *C. longifolia* flowers slightly later than *A. morio*, the slopes of the regression models for the two species were statistically similar ($F_{1,176} = 1.3364$, $P = 0.249$). As expected, given the separation in flowering period, the elevations were statistically dissimilar ($F_{1,177} = 86.778$, $P < 0.001$).

Figure 3-10. Relationship between flowering date (days after 1 March) and mean spring (March – May) temperature for *Platanthera chlorantha* ($y = 145.1 - 4.687x$, $r^2 = 0.083$, $P < 0.001$, $n = 109$) and *P. bifolia* ($y = 152.6 - 5.349x$, $r^2 = 0.14$, $P < 0.001$, $n = 110$). Symbols: ● = data for *P. bifolia*; ● = data for *P. chlorantha.*
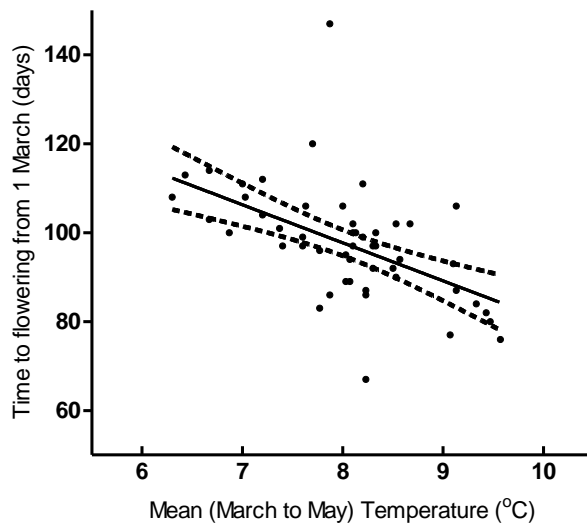
Figure 3-11. Relationship between flowering date (days after 1 March) and mean spring (March – May) temperature for *Cephalanthera longifolia* ($y = 166.3 - 8.566x$, $r^2 = 0.30$, $P < 0.001$, $n = 54$).

For the nectar-rewarding species pollinated by Lepidoptera there was striking similarities between the 2 closely related species of the genus *Platanthera*. For *P. chlorantha* the regression of flowering date on mean spring temperature accounted for 8.3% of the variation in flowering time (Fig 3-10). A 1°C increase in mean spring temperature was associated with an advance in flowering of 4.7 days. For *P. bifolia* linear regression of flowering date on mean spring temperature accounted for 14% of the variation in flowering time (Fig. 3-10) and a 1°C increase in mean spring temperature was associated with an advance in flowering of 5.3 days. The slopes ($F_{1,215} = 0.1132$, $P = 0.737$) and intercepts ($F_{1,216} = 1.526$, $P = 0.218$) of the regression models were statistically indistinguishable, demonstrating a common phenological response to spring temperature.

Within the group pollinated by Lepidoptera, the non nectar-rewarding *Anacamptis pyramidalis* flowers later in the season than the rewarding *P. chlorantha*. For *A. pyramidalis* the regression of flowering date on mean spring temperature (Fig. 3-12) accounted for 15% of the variation in flowering time. A 1°C increase in mean spring temperature was associated with an advance in flowering of 7.1 days. As expected,
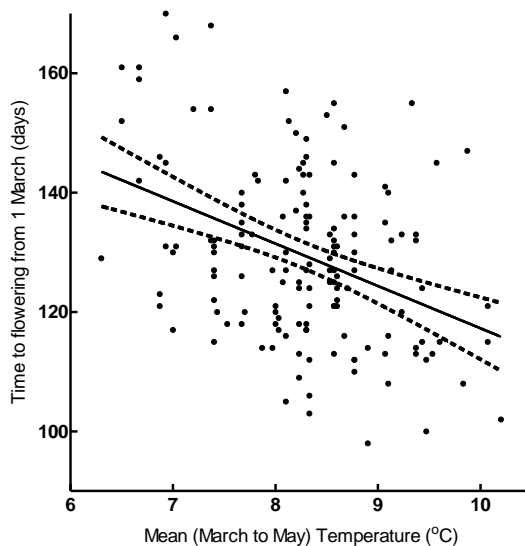
Figure 3-12. Relationship between flowering date (days after 1 March) and mean spring (March – May) temperature for *Anacamptis pyramidalis* ($y = 188.3 - 7.10x$, $r^2 = 0.15$, $P < 0.001$, $n = 151$).

given the separation in flowering period, the elevations were statistically dissimilar ($F_{1,257}$ = 182.484, P< 0.001). Nonetheless slopes of the regression models for the two species were statistically similar ($F_{1,256}$ = 1.3833, P = 0.241), showing a shared flowering response to spring temperature.

Neotinea ustulata flowers earlier than *Dactylorhiza praetermissa* and both are non nectar-rewarding pollinator generalists. For *D. praetermissa* the regression of flowering date on mean spring temperature (Fig. 3-13a) accounted for 6.9% of the variation in flowering time. A 1°C increase in mean spring temperature was associated with an advance in flowering of 4.2 days. For the earlier flowering *N. ustulata* the regression of flowering date on mean spring temperature (Fig. 3-13b) accounted for 9% of the variation in flowering time. A 1°C increase in mean spring temperature was associated with an advance in flowering of 4.1 days. The slopes of the regression models were statistically similar ($F_{1,349}$ = 0.0091, P = 0.924), while as predicted the elevations were statistically dissimilar ($F_{1,350}$ = 92.726, P< 0.001).

Within the group of non nectar-rewarding pollinator generalists, *Dactylorhiza fuchsii,* flowering later than *D. praetermissa*, demonstrated a significant but relatively weak correlation to spring temperature. The regression of flowering date on mean spring temperature (Fig. 3-14) accounted for only 3% of the variation in flowering time. A 1°C increase in mean spring temperature was associated with an advance in flowering of 2.2 days. The slopes of the regression models for the two species were not statistically different ($F_{1,443}$ = 2.1207, P = 0.146). As expected, given the separation in flowering period, the differences between the intercepts were statistically significant ($F_{1,444}$ = 54.773, P< 0.001).

The two nectar-rewarding orchids pollinated by Hymenoptera, *Epipactis purpurata* and *Spiranthes spiralis* are late-season species, flowering into the autumn. Even so linear regression models of flowering time on spring temperature gave results consistent with earlier flowering orchid species. For *E. purpurata* linear regression of flowering date on mean spring temperature accounted for 15.9% of the variation in flowering time (Fig. 3-15), and a 1°C increase in mean spring temperature was associated with an advance in flowering of 7.5 days. For *S. spiralis* linear regression on flowering date accounted for 12.5% of the variation in flowering time (Fig. 3-15),

and a 1°C increase in mean spring temperature was associated with an advance in flowering of 5.4 days. The slopes of the regression models for the two species were statistically indistinguishable ($F_{1,200} = 0.8603$, $P = 0.355$) .The intercepts were statistically dissimilar ($F_{1,201} = 125.236$, $P < 0.001$) presumably reflecting the separation in time of onset of flowering.
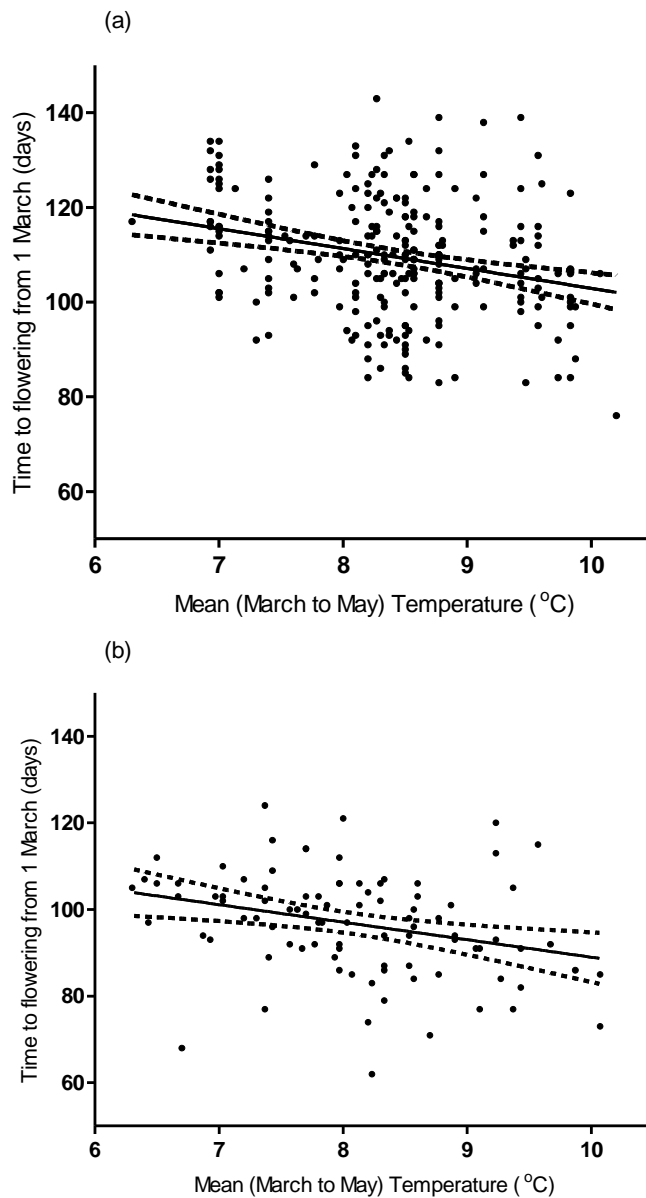
Figure 3-13. Relationship between flowering date (days after 1 March) and mean spring (March – May) temperature for (a) *Dactylorhiza praetermissa* ($y = 145.0 - 4.207x$, $r^2 = 0.07$, $P < 0.001$, $n = 261$) and (b) *Neotinea ustulata* ($y = 129.4 - 4.045x$, $r^2 = 0.09$, $P < 0.01$, $n = 92$).
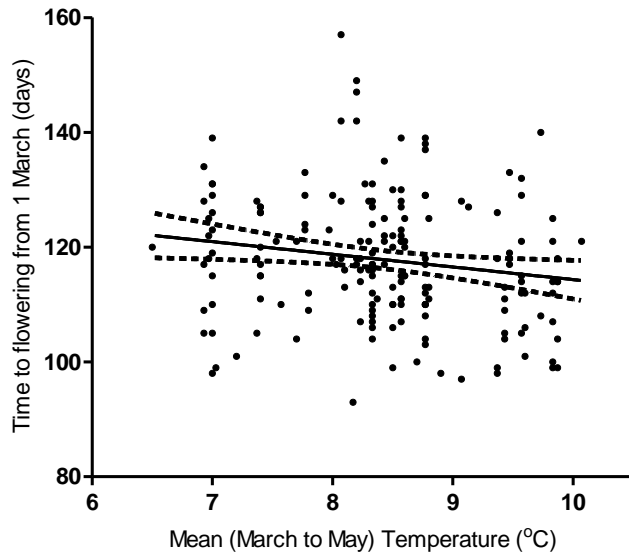
Figure 3-14. Relationship between flowering date (days after 1 March) and mean spring (March – May) temperature for *Dactylorhiza fuchsii* ($y = 136.4 - 2.206x$, $r^2 = 0.03$, $P < 0.05$, $n = 186$).

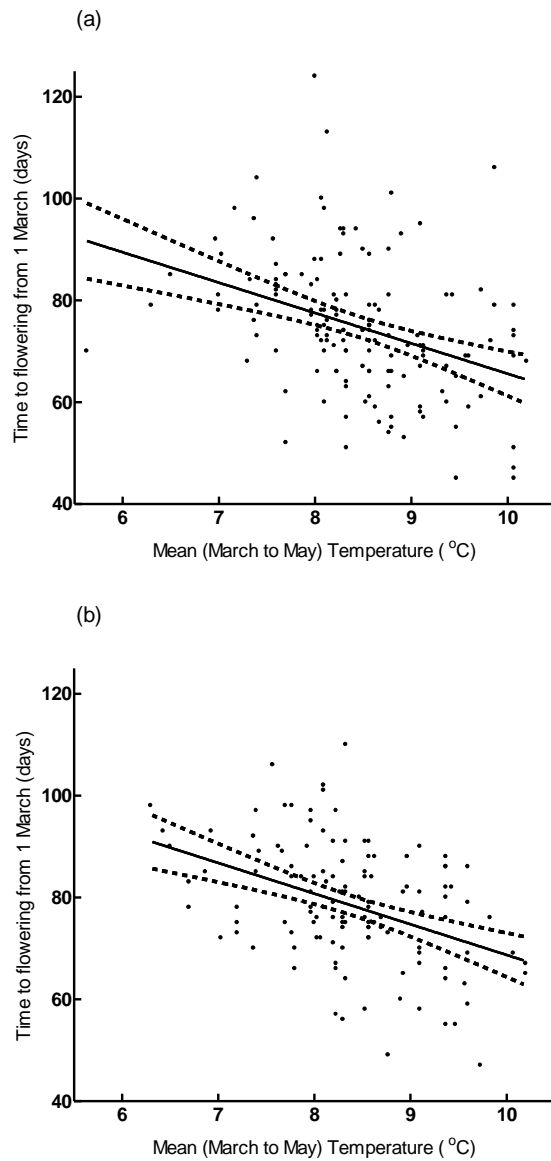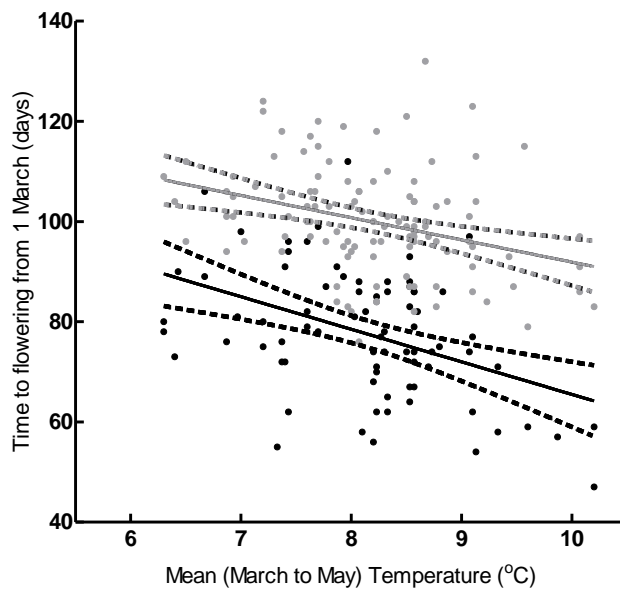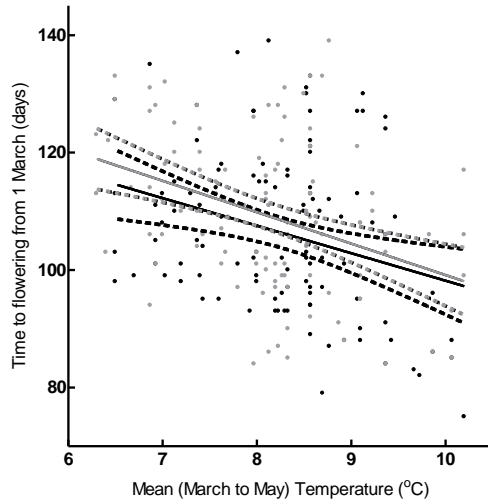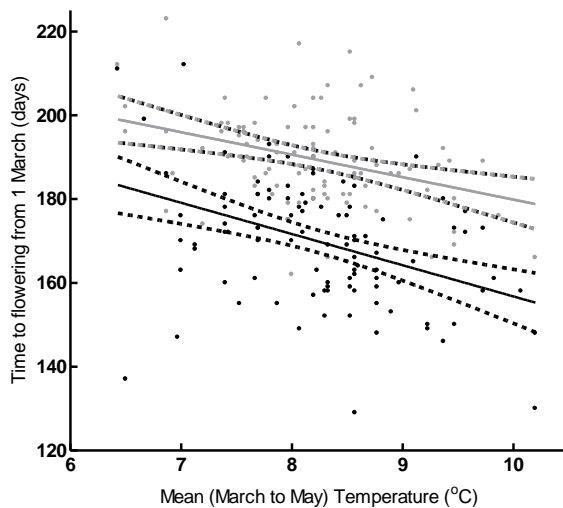**Figure 3-15.** Relationship between flowering date (days after 1 March) and mean spring (March – May) temperature for the late-flowering species; *Epipactis purpurata* ($y = 231.3 - 7.45x$, $r^2 = 0.159$, $P < 0.001$, $n = 104$) and *Spiranthes spiralis* ($y = 233.5 - 5.364x$, $r^2 = 0.125$, $P < 0.001$, $n = 100$). Symbols: ● = data for *E. purpurata*; ● = data for *S. spiralis*.

Of the 13 orchid species for which it was possible to test flowering responses to mean spring temperature, the responses of the 3 non nectar-rewarding pollinator generalists were the most muted: flowering advanced between 2.2-4.2 days per 1°C increase in mean spring temperature (Table 3-9). It was not possible to perform this test for the sole nectar-rewarding pollinator generalist, *Neottia ovata*. Even so, the response of *D. praetermissa*, a non-rewarding pollinator generalist, was statistically identical to the response demonstrated by the rewarding Lepidoptera pollinated species, *P. bifolia*. These species flower at the same time and tolerate similar habitat. The slopes ($F_{1,367}$ = 0.5154, P = 0.4733) and intercepts ($F_{1,368}$ =1 5195, P= 0.2185) of the regression models for the two species were statistically indistinguishable, suggesting a shared response to spring temperature.

For *D. fuchsii*, mean summer (June-August) temperature was most significant in flowering response to temperature (Table 3-6), and two further mid to late-flowering species, *E. purpurata* (Table 3-4), and *A. pyramidalis* (Table 3-5), demonstrated highest correlations of peak flowering time with mean summer (June-August) temperature. These species were drawn from each of the 3 designated pollinator groups: Hymenoptera, Lepidoptera and pollinator generalist. For the nectar-rewarding Hymenoptera pollinated orchid, *E. purpurata* linear regression on mean June-August temperature accounted for 31% of the variation in flowering time, and a 1°C increase in mean spring temperature was associated with an advance in flowering of 10 days. Similarly for the non nectar-rewarding, Lepidoptera pollinated species, *A. pyramidalis,* linear regression on mean June-August temperature again provided the best fit, accounting for 17% of the variation in flowering time, and a 1°C increase in mean spring temperature was associated with an advance in flowering of 8.5 days.

The slopes ($F_{1,251}$ = 0.4463, P = 0.505) of the regression models for the two species were statistically indistinguishable. The intercepts, however, were statistically dissimilar ($F_{1,252}$ = 570.806, P< 0.001), reflecting the difference in onset of flowering.

For *D. fuchsii*, a non nectar-rewarding pollinator generalist, mean summer temperature accounted for 8% of the variation in flowering time, and an increase of 1°C in mean summer temperature was associated with an advance in peak flowering of 4.3 days. The flowering response to mean summer temperature of *D. fuchsii* and the

rewarding Hymenoptera pollinated *E.purpurata*, were dissimilar: the differences between the slopes were significant ($F_{1,286} = 9.9580$, $P = 0.002$).

3.4 Discussion

The 15 orchid species in this study comprise some of the most well-known species native to the UK and represents approximately 30% of the total number of species present in the British Isles. They are drawn from three pollination groups and a progression of flowering dates. This study, using historical climate records and herbarium specimens collected over a period of 164 years between 1823 and 1986 represents the first study of flowering phenology for a series of British orchid species, and establishes the importance of spring temperature in determining flowering time.

Recently herbarium specimens have been used to investigate flowering phenology for a range of species (Primack *et al.* 2004; Miller-Rushing *et al.* 2006; Gallagher, Hughes & Leishman 2009; Molnár *et al.* 2012b; Panchen *et al.* 2012) and, their value as proxy for field observation has been validated explicitly using herbarium and field data for the orchid *Ophrys sphegodes*, in the UK (Robbirt *et al.* 2011). (Full details can be found in Chapter 2).

Whilst collection activity varied between species, two clear periods of intense collection were apparent: during the 1860s to 1880s and again during the 1930s to 1950s. Collection activity declined dramatically after 1960 and, for the species in this study, no specimens were collected after 1986. This decline in collection activity reflects the rise in awareness of the negative impact for conservation of unrestricted collecting, and the protection of rare species through legislation. This pattern of collection activity is rather earlier than a comparable analysis of orchid collection in Hungary, where peak collection was clearly set in the 1950s (Molnár *et al.* 2012a). In that study close to 90% of the specimens were accurately dated, which is considerably more than the 60% of fully dated specimens found in this research. The difference in wastage almost certainly reflects the difference in age profiles of the collections. Accuracy of dating improved considerably during the 1900s, and the majority of incompletely referenced and undated specimens in this study originated in the 1800s.

Peak flowering is expected to be a more reliable indicator of flowering phenology than first-flowering (Miller-Rushing, Inouye & Primack 2008) given the possibility of undue influence of stochastic events in first flowering dates (Sparks & Carey 1995). Despite the possibility of bias which could result from variation in collection effort and confound the detection of underlying trends (Case *et al.* 2007), herbarium specimens would, on average, be expected to be collected at peak flowering time and this expectation has been borne out in this study since just 6% of specimens were discarded because flowers were senescing or in seed or less than 40% of flowers were in bloom. Further, herbarium specimens in this research show accurate representations of accepted flowering periods.

Thirteen of the 15 orchid species (87%) demonstrated a significant correlation with mean spring (March-May) temperature: *Gymnadenia conopsea* and *Neottia ovata* were the exceptions. A low signal to noise ratio is not unexpected for either of these species given the wide variation in flowering range, and for *N. ovata* any signal is likely to be further confounded by its occurrence across a particularly broad habitat range. Flowering date of spring and early summer flowering orchid species were most strongly correlated with temperatures over the three to four months immediately prior to flowering. For those species which flowered later in the season, seasonal temperature over spring and summer were both highly correlated with flowering time, and for three late-flowering species mean summer temperature was the most significant variable.

These findings are similar to those of Fitter *et al*. (1995) who, in a long-term field study of first flowering date for 243 species located at a single site in Central England between 1954 and 1989, found that the majority of species flowering between January and April were most significantly associated with mean temperature in the one or two months immediately prior to flowering. In the same study a more even response to temperature was demonstrated for species flowering during the summer months, when temperature over the four months prior to flowering was important. Prior studies across diverse taxonomic plant groups have relied on averaged results due to the considerable differences between species (Fitter *et al.* 1995; Sparks, Jeffree & Jeffree

2000). This study has demonstrated a coherent phenological response for a group of plant species within the same taxonomic family.

Geographical variation in collection site was significantly correlated with flowering date for several orchids in this study. A geographic effect would be expected, especially for those species collected over a wider area. In all species, controlling for the effects of latitude and longitude had only a marginal effect on absolute correlation values between flowering date and mean spring or summer temperatures, and had no effect on their significance.

The effect of latitude and longitude was similar for *Platanthera bifolia* and *Platanthera chlorantha* - both demonstrated later flowering with northing and with westing. During summer months western regions of the British Isles are wetter and cooler than eastern regions. *Platanthera bifolia* and *P. chlorantha* are summer flowering species and would be expected to flower earlier in the east in response to higher temperatures. The apparent geographic effect is not straightforward, however, and would be influenced by multiple factors including habitat preference, micro-climate and distribution patterns. *Platanthera bifolia* has a northerly and westerly tendency in its distribution pattern, and favours damp heathland and moorland, bogs, woodland and rough pasture. There are purportedly two forms of the species distinguished by habitat preference, the commoner damp heathland and moorland form and the scarcer woodland form. The heathland form of southern and eastern England has suffered severe population decline and has been lost from much of the region since the 1970s, chiefly due to habitat loss. The moorland form of the north and west regions have suffered a less severe fall in population levels. Change in distribution pattern of the heathland form since the 1970's post-date the period of herbarium specimens in the dataset, and thus cannot be an explanatory factor in the observed geographic effect. The woodland form is restricted to southern England where it favours open deciduous woodland, often beech, over calcareous soils. Since the woodland form flowers slightly earlier (May-June) than the heathland form (June-July) this may be a contributory factor in the earlier flowering observed toward the east. *Platanthera chlorantha* has a southern bias to its distribution pattern and preferring dappled shade it is often found at woodland margins or in clearings. Further north it is most usually

found in tall grassland of pastures and meadows and is reported to flower up to 3-4 weeks later than the south. Flowering would be expected to be earlier in the east in response to higher summer temperatures.

In stepwise forward regression mean monthly temperature in March, April or May was flagged as a key variable for every orchid species irrespective of flowering time, underlining the impact of spring temperature in relation to flowering phenology.

In linear regression of flowering response to mean spring temperature there were no significant differences in flowering response for twelve (92%) of the thirteen orchid species for which linear regression could be applied; flowering response of *D. fuchsii* being the exception. Two species, *Gymnadenia conopsea* and *Neottia ovata*, did not present a significantly non-zero relationship with mean spring temperature and were thus excluded from the analysis.

For 12 of the 15 orchid species (80%) in this study flowering advanced between 4.2 and 8.6 days for each 1°C increase in mean spring temperature. These findings are strikingly similar to those of British studies of first flowering dates based on observational records. Fitter *et al*. (1995) reported a mean advance of 4.4 days per 1°C rise in spring temperature. Sparks *et al*. (2000) established an advance in mean flowering of 24 species averaged across the UK of 2 to 10 days per 1°C rise in temperature. Findings from these British studies are also in line with observational studies based in Central Europe (Walkovszky 1998; Menzel, Estrella & Fabian 2001) and North America (Shetler & Wiser 1987; Bradley *et al.* 1999).

Fourteen of the 15 species in this study showed no significant associated with mean temperature in the autumn prior to flowering. *Ophrys insectifera* was the sole exception, demonstrating a significant negative correlation with mean autumn temperature of the prior year. Similarly 12 of the 15 species in this study did not show a significant correlation with mean precipitation of the previous autumn. *Anacamptis morio* and *Cephalanthera longifolia* both presented significant positive correlations with mean precipitation for autumn of the prior year, whilst *Epipactis purpurata* demonstrated a significant negative correlation. These findings are broadly consistent with the findings of prior studies of Sparks & Carey (1995) and Sparks, Jeffree &

Jeffree (2000) who found that associations with autumn temperature, where present, were small and usually positively associated with flowering date. Overall climate of the prior year has little influence on flowering phenology (Sparks & Carey 1995). Fitter *et al.* (1995) found that first flowering date of spring flowering plants was influenced to some extent by temperature of the prior autumn but did not quantify the relationship.

Fitter (1995) found that insect-pollinated plant species were more responsive to warming than wind-pollinated species and Molnar reported that on average, non-rewarding orchids varied more over time than rewarding (Molnár *et al.* 2012b). For the orchid species in this study, all insect-pollinated, there was no evidence that flowering response to spring or summer temperature was influenced by whether or not reward was offered, and there were no differences between the responses of food deceptive or sexually deceptive orchids. Linear regression of flowering date (days from 1st March) on mean spring temperature were statistically alike both in comparison of rewarding and non-rewarding species, and in comparison of species from contrasting pollinator groups. These results suggest a common phenological response to temperature across the orchid species in this study.

For those species which rely on a specific pollen vector, such as the sexually deceptive orchids *Ophrys sphegodes* and *Ophrys insectifera*, pollination success is critically dependent on synchrony of peak flowering time of the orchid with peak flight time of the pollinator. The potential for asynchrony between plant and pollinator under current climate warming should be evaluated at the species level, especially for non-rewarding species where convergent selection pressure is absent. The potential for asynchrony between orchid species and pollen vectors is examined in Chapters 4 and 5.

The lack of long-term observational data of flowering time has previously hindered rigorous phenological study of plant species both in the UK and elsewhere. Nevertheless the associations between climate warming and plant phenologies needs to be understood at the species level as a prerequisite to investigation of the potential consequences of climate warming on ecological interactions on a wider scale. Although prior studies of temperate orchid ecology have established that a complex interaction of variables impact flowering performance (Hutchings 2010) and

phenology (Molnár *et al.* 2012b) none has previously quantified the relationship between flowering phenology and climatic variables. Using historical climate data and herbarium specimens collected through the 19th and 20th centuries his research represents a novel investigation into the flowering phenology of a range of temperate orchid species native to the British Isles. The results of this research demonstrate a broadly consistent flowering response to spring temperature within the wide range of orchids in this study. All the species in this study advanced flowering in response to higher spring temperature, and for 13 of the 15 species the advance was significant. Strong responses to spring temperature were seen in all species within the groups pollinated by Hymenoptera, by Lepidoptera and the two sexually deceptive species. Generally weaker responses were seen in those species grouped as pollinator generalist. Even so, comparing specific species, responses were statistically identical across nectar-rewarding, sexually deceptive, and non nectar-rewarding species.

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Chapter 4

Phenological responses of sexually deceptive orchids and their pollinators to temperature: implications of climate change for the maintenance of synchrony

Summary

1. Although direct long-term observations for the detection of phenological trends in response to climate change are not available for most species, dated plant specimens in herbaria have been shown to provide a reliable proxy. This should also be true for museum collection of other organisms. Parallel records of phenological responses in different species could be used to predict the potential for disruption of relationships between species as a result of climate change. Many orchids have highly evolved, specific pollination systems that rely on synchrony of flowering with insect activity, and therefore may be susceptible to disruption due to climate warming. This research investigated records for two orchids that employ sexual deceit to attract male insects to attempt copulation with the flower (*Ophrys sphegodes* and *O. insectifera*) and their respective pollinators (the solitary bee *Andrena nigroaenea* and solitary digger wasp *Argogorytes mystaceus*).

2 Relationships between collection date of *Andrena nigroaenea* and various measures of mean spring temperature were examined using 2,980 field observations of flying time between 1975 and 2009 and 357 museum specimens collected between 1893 and 2007. These were compared with the known trend in flowering time of *O. sphegodes* in response to mean spring (March-May) temperature. Similar comparisons were made using 657 field records (1891-2010) and 129 museum specimens (1897-2006) of *Argogorytes mystaceus* and collection time of 114 herbarium specimens of *O. insectifera* (1835-1970).

3. A 1°C rise in mean spring (March-May) temperature was associated with an overall advance in flight time of *Andrena nigroaenea* of 7-11 days, compared with an advance in flowering time of 6 days for *O. sphegodes*. However male bees emerged earlier than females on average and responded to temperatures earlier in the spring. In comparison, a 1°C rise in mean spring (March-May) temperature was associated with an advance in

flowering of *c*. 4 days in *O. insectifera* and an advance in flight of *c.* 6 days in *Argogorytes mystaceus.* Males and females of *A. mystaceus* were distinguished in the museum record but there were no significant difference in response between the sexes.

4. Flight dates of males and females of *A. nigroaenea*, distinguished in the museum record, were compared with predicted flowering dates for *O. sphegodes*. The proportion of years with a mean date of male flight on or before predicted peak flowering increased with mean spring temperature over the range 7-10°C.

In years where both sexes were collected, the proportion of females collected on or before predicted flowering time also increased with temperature. In years where one or other sex was collected before predicted flowering date, the probability of it being female similarly rose with increasing temperature.

5 Whilst there is no evidence of potential asynchrony between flowering and flight of *O. insectifera* and *A. mystaceus*, for *O. sphegodes* there is considerable potential for phenological divergence between flowering and flight of the male bees of *A. nigroaenea* with increasing temperature, and disruption to pollination may be augmented by the earlier emergence of females.

4.1 Introduction

The influence of climate on the timing of seasonal and developmental events has been reported across a range of organisms, including birds (Crick *et al.* 1997), insects (Sparks & Yates 1997), amphibians (Beebee 1995), plants (Sparks & Carey 1995; Fitter & Fitter 2002) and fungi (Kauserud *et al.* 2008). Long-term datasets are needed to detect phenological trends (Sparks 2007; Magurran *et al.* 2010; Bolmgren, Vanhoenacker & Miller-Rushing 2012), as they may be obscured by inter-annual or decadal variation (Badeck *et al.* 2004; Bolmgren, Vanhoenacker & Miller-Rushing 2012). Direct long-term observations are rarely available (Sparks & Carey 1995; Holopainen *et al.* 2012) and for most part they are concerned with recording the commencement of a seasonal event (Bolmgren, Vanhoenacker & Miller-Rushing 2012), rather than central (peak) values, which should be more reliable for phenological study (Miller-Rushing, Inouye & Primack 2008). Recently, both problems have been addressed by the use of specimen-based records as a source of phenological data (Primack *et al.* 2004; Lavoie & Lachance 2006; Gallagher, Hughes & Leishman 2009; Panchen *et al.* 2012). In particular, the collection date for herbarium specimens on average represents peak flowering and their use for as proxy for field observation of peak flowering date has recently been validated (Robbirt *et al.* 2011).

Asynchrony between phenophases may occur where species respond differentially to climate change (McCarty 2001; Stenseth & Mysterud 2002; Stenseth *et al.* 2002); given that species clearly do show unique phenological responses to climate warming (Abu-Asab *et al.* 2001; Fitter & Fitter 2002; Miller-Rushing & Primack 2008) there is potential for disruption of ecological interactions (Walther *et al.* 2002; Parmesan 2006; Thackeray *et al.* 2010).

Detailed knowledge, however, is needed of phenological relationships at species level in order to understand these community consequences of climate-driven phenological shifts (Bolmgren, Vanhoenacker & Miller-Rushing 2012; Nakazawa & Doi 2012). To date there have been few studies of the potential for temporal disruption in plant-pollinator relationships, and results have been varied: some report potentially

asynchronous outcomes (Doi, Gordo & Katano 2008; Pradal, Olesen & Wiuf 2009), whilst others report parallel temporal responses (Bartomeus *et al.* 2011).

Ideal models of plant-pollinator synchronicities are found in highly co-evolved insect pollination systems, as exemplified in the Orchidaceae; the largest family of flowering plants. Cross-pollination via sexual deceit is highly specific, and frequently reliant on one or two closely related pollinator species (Schiestl *et al.* 2000). In sexual deception orchid flowers have evolved visual and olfactory characteristics that attract male insects to attempt copulation, thereby achieving pollination. Seed production of orchids is generally pollinator-limited (Nilsson 1992; Neiland & Wilcock 1995; Tremblay *et al.* 2005) and divergence between the flying time of the pollinating insects that are the subject of sexual deception and the flowering time of the orchids would impair pollination success.

The genus *Ophrys* is representative of sexually deceptive orchids in Europe. *O. sphegodes* is pollinated almost exclusively by the solitary bee *Andrena nigroaenea* (Ayasse *et al.* 2000; Schiestl 2005)*,* and *O. insectifera* by the wasp *Argogorytes mystaceus* (Borg-Karlson 1990; van der Cingel 1995). As is the case for most insects, sets of data recording flying time and associated climatic variables have not been assembled until recently for either insect, and the specimen-based records that could potentially provide a longer perspective on the effects of climate on flying time have not yet been used for this purpose. In addition to understanding how climate change may affect the synchronicity between insect and orchid, however, it is important to know whether there are differences in the phenological responses of male and female insects, since females are stronger competitors with the orchid flowers for the copulatory attentions of the males (Nilsson 1992).

The aims of this work were to compare the phenological responses to spring temperature of flowering in *O. sphegodes* and *O. insectifera* with the equivalent changes in flying time of their respective pollinators, males of *Andrena nigroaenea* and *Argogorytes mystaceus*, and investigate the potential for loss of synchrony between them with increasing spring temperature. A fundamental hypothesis was that dated museum specimens would represent a verifiable, long-term record of insect flying time, much as herbarium specimens can be used to represent peak flowering

time in plants (Robbirt *et al.* 2011). Specifically, this work sought to (*i*) examine museum records for *Andrena nigroaenea* and *Agogorytes mystaceus* in order to determine flying-time trends in relation to temperature; (*ii*) compare climate-related trends with equivalent ones that could be detected from more recent field-based observations of flying time; (*iii*) investigate whether there are differences in the behaviour of male and female insects, using the museum-based collections; (*iv*) examine herbarium records of *O insectifera* to determine whether there are similar phenological trends in flowering time associated with spring temperature to those already demonstrated for *O. sphegodes* (Robbirt *et al.* 2011); and (*v*) predict peak flowering times for both orchids over a range of spring temperatures and relate these to the flying times of male and female insects.

4.2 Methods

4.2.1. The study species

4.2.1.1 The Ophrys sphegodes-Andrena nigroaenea-*pollination system*

Chapter 2, Section *2.2.1* provides a full description of *Ophrys sphegodes* and an account of the pollination system.

The solitary mining bee *Andrena nigroaenea*, (Apidae, Andreninae), is the primary pollinator of *Ophrys sphegodes* subsp. *sphegodes* (Ayasse et al. 2000; Paxton & Tengö 2001; Mant, Peakall & Schiestl 2005). Whilst *A. barbilabris*, *A. thoracica*, *A. cineraria* and *A. limata* have also been suggested as secondary pollinators (Gaskett 2011), their effectiveness has not been established.

The male bees emerge a few days before the female bees; males significantly outnumber females and rely on non-territorial 'scramble competition' to find mates (Paxton 2005). The orchid flower, resembling the female bee, and flowering in synchrony with the emergence and flight of the male bee, attracts newly emerged males to attempt copulation. Both visual and chemical mimicry are utilised; the flower, mimicking the body of a female bee, emits a floral scent almost identical to the sex pheromone of the receptive female (Schiestl *et al.* 2000). Pollinated flowers emit an altered floral odour (Schiestl & Ayasse 2001) which deters further copulatory attempts by the male bee thereby increasing the probability of male bees attempting

pseudocopulation with other flowers. Consequently the phenology of the male bees is important both in relation to flowering time and to the phenology of the female bees, which represent a rival attraction for the attention of male bees.

A. nigroaenea is a solitary mining bee (Archer 2004) that is distributed widely across Europe but in Britain it is predominantly recorded from central and southern regions (Edwards 2011). It is not currently regarded as rare in Britain and is found across a range of habitats (Edwards 2011). The female nests singly in short turf or bare ground, favouring light, sandy soils (Edwards 2011). Each larva hatches and develops within its individual chamber, over-winters as a pupa and is one of the earliest of the bee species to emerge in spring. Males are relatively small and outnumber the females, which can be distinguished primarily by the distinctive orange pollen hairs of their hind legs. The flight period in Britain is essentially from the beginning of April to late June, with males habitually reported as patrolling 'rendez-vous' sites in search of mates. The species is univoltine, although since the 1990s there have been some reports in the UK of a putative partial second generation, presumably arising from longer warm periods (Edwards 2011).

4.2.1.2 The Ophrys insectifera-Argogorytes mystaceus *pollination system*

Chapter 3, Section *3.2.2.1.2* provides a full description of *Ophrys insectifera* and an account of the pollination system.

Argogorytes mystaceus (Apoidea, Sphecidae) is a solitary digger wasp (Archer 2004). Although it is present throughout the British Isles, favouring woodland edges and clearings, it follows a marked southerly distributional cline and is relatively scarce across northern England and Scotland (Richards 1980; Allan 2001). The species is univoltine, typically flying between April and June, exceptionally to September (Richards 1980; Allan 2001). Nests, dug in dry banks, consist of a vertical to a depth of approximately 10cm. The burrow then branches along a horizontal plane to form several cells. The female lays a single egg in each cell, provisioning each with paralysed nymphs of the spittle bug, principally *Philaenus spumarius* (Allan 2001). Males are important pollinators of *O. insectifera*, and are thought to seek out the flowers because of the close resemblance of the flower scent to the sex pheromone of the female wasp (Allan 2001).

4.2.2 Museum and herbarium data

4.2.2.1 Ophrys sphegodes

Robbirt *et al.* (2011) (Chapter 2) established the relationships between peak flowering time in *Ophrys sphegodes* and mean spring (March-May) temperature for data derived from two sources: 77 fully dated herbarium specimens collected between 1848 and 1958 and stored in the herbaria of the British Museum and the Royal Botanic Gardens, Kew and field observations made between 1975 and 2006 of the date of peak flowering for a population of *O. sphegodes* at Castle Hill National Nature Reserve, Sussex, UK. The relationships obtained, both for date of peak flowering and the relationship between date of peak flowering and mean spring temperature were statistically indistinguishable for the two datasets. Therefore, the herbarium and field records have been combined into a single dataset containing 102 records of peak flowering date, with at least one data point in each of 82 years during a 159-year period from 1848 to 2006. The date of peak flowering is expressed here as days after 1 March in order to facilitate comparison with the data for flying date of the pollinator, *Andrena nigroaenea*.

4.2.2.2 Ophrys insectifera

All 285 specimens of *Ophrys insectifera* held in herbaria at the Natural History Museum, London (BM, 210 specimens) and Royal Botanic Gardens, Kew (K, 75 specimens) were examined. Specimens with incomplete data for time and place of collection were discarded. In order to ensure the dataset represented peak flowering as far as possible specimens with withered flowers or in fruit, and those with less than 40% open flowers were rejected. One hundred and fifty five (54%) of the specimen were discarded as undated or illegible. Of those remaining 7 were in seed and 9 were less than 40% open. The final dataset comprised 114 specimens, providing at least one data point for each of 72 years, spanning a 136 year period from 1835 to 1970. Vice county centroids of collection ranged from -2.7610 to 0.9119 decimal longitude (range 3.6729 decimal degrees), and from 50.6758 to 54.3931 decimal latitude (range 3.7173 decimal degrees).

4.2.2.3 Andrena nigroaenea

Eight hundred and forty eight British specimens of *A. nigroaenea* held at the Natural History Museum, London (BM, 625 specimens) and Oxford University Museum of Natural History (OUM, 223 specimens) (Fig.4-1a,b) were examined. Of these 293 (34.6%) of the specimens were rejected as undated or unable to be read. Of those remaining 179 were redundant, multiple collections (clearly collected on the same day at the same location), 12 were of unknown location, 3 could not be reliably sexed and 4 originating from Wales were discarded as geographical outliers. The final dataset comprised 357 specimens, providing at least one data point for each of 81 years, spanning a 115-year period from 1893 to 2007. Whilst collection activity was greatest during the 1920s to 1940s, the overall spread of activity was fairly equitable until the 1960s, after which collection declined considerably (Fig. 4-3a). Vice county centroids of collection ranged from -3.0746 to 1.5279 decimal longitude (range 4.603 decimal degrees), and from 51.3658 to 55.2198 decimal latitude (range 3.854 decimal degrees).

4.2.2.4 Argogorytes mystaceus

Four hundred and eighteen British specimens of *A. mystaceus* held at the Natural History Museum, London (BM, 333 specimens), Oxford University Museum of Natural History (OUM, 41 specimens) (Fig.4-2a, b) and University Museum of Zoology Cambridge (UMZC, 44 specimens) were examined. Of these 251 (60%) were rejected as undated or illegible, 20 were redundant multiple collections, 10 representing geographical outliers from Scotland or Wales, 4 of unknown location and 4 that could not be reliably sexed. The final dataset comprised 129 specimens, providing at least one data point for each of 59 years, spanning a 128-year period from 1879 to 2006. Peak collection activity was during the 1920s to 1950s, with a sharp decline after 1960 (Fig. 4-3b). Vice county centroids of collection ranged from -4.6080 to 1.3418 decimal longitude (range 5.950 decimal degrees), and from 50.5040 to 54.3931 decimal latitude (range 3.889 decimal degrees).

(a)   (b)

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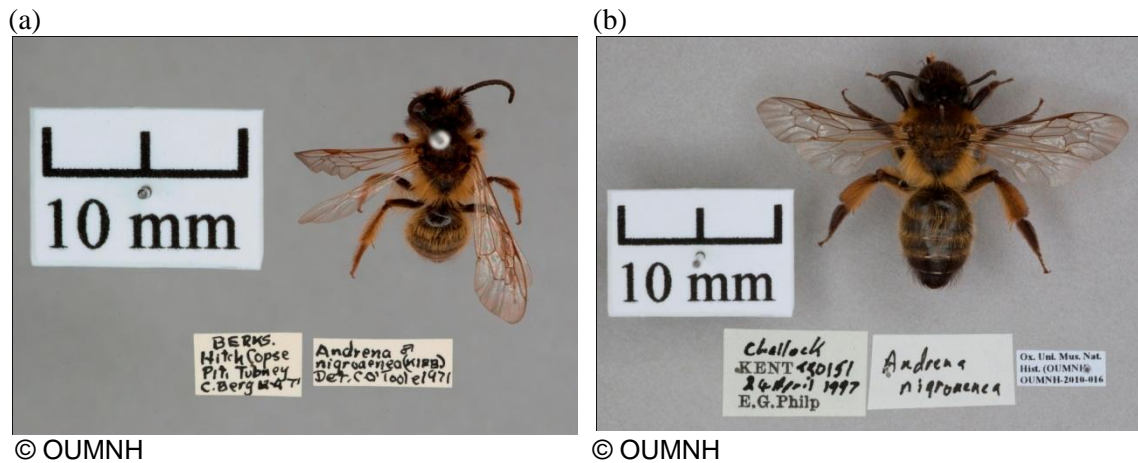
Figure 4-1. Specimens of *Andrena nigroaenea* held at Oxford University Museum of Natural History: (a) male collected by C.Berg at Hitch Copse, Tubney, Berkshire on 12 April 1971 and (b) female collected by E.G.Philp at Challock, Kent on 24 April 1997.

(a)   (b)

© OUMNH   © OUMNH
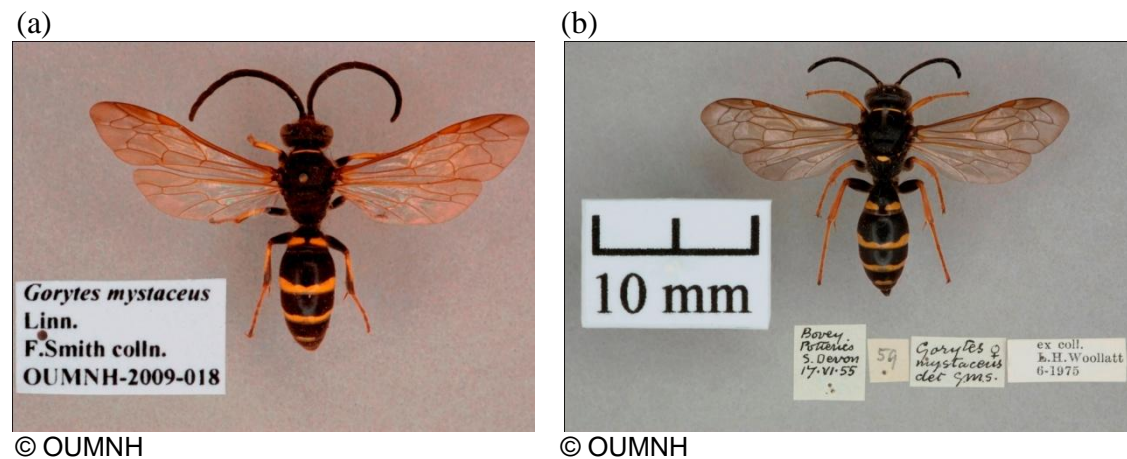
Figure 4-2. Specimens of *Argogorytes mystaceus* held at Oxford University Museum of Natural History: (a) male collected by F. Smith (date and location unknown), and; (b) female collected at Bovey Potteries, South Devon on 17 June 1955.

4.2.3 Field observations of insects

4.2.3.1 Andrena nigroaenea

All 3,696 British observation records of *A. nigroaenea* supplied by the Bees Wasps and Ants Recording Society (BWARS) were examined. Of these, 165 records were inadequately dated and 192 were from Wales or Scotland and thus outside the geographic range of the temperature data: these were discarded. The remainder were

heavily skewed towards recent (1975-2009) records (Fig. 4-3a) and so for homogeneity only the 2,997 observations (90%) made from 1975 to 2009 were selected.

Redundant multiple observations (17), clearly collected on the same day and from the same location were included as a single data point, giving a final dataset of 2980 observations recorded between 1975 and 2009, and enabling direct comparison with contemporary (1975-2006) field records of peak flowering of *Ophrys sphegodes* (Robbirt *et al.* 2011). Vice county centroids of the BWARS observations ranged from -5.26012 to 1.34183 decimal longitude (range 6.602 decimal degrees) and 50.21018 to 55.50709 decimal latitude (range 5.297 decimal degrees).

4.2.3.2 Argogorytes mystaceus

All 1,005 British BWARS records of *A. mystaceus* were examined. Of these, 81 were discarded as insufficiently dated, as were a further 112 from Wales and Scotland and 148 redundant multiple observations. Eight late-season observations were also rejected, representing outliers recorded more than 164 days from 1 March: these fell well outside of the customary flight period of the species and could not be verified. This provided a final dataset of 657 observations recorded between 1891 and 2010 (Fig. 4-3b). Vice county centroids of the BWARS observations ranged from -5.2601 to 1.3418 decimal longitude (range 6.602 decimal degrees) and 50.2102 to 54.7254 decimal latitude (range 4.515 decimal degrees).

4.2.4 Meteorological data

Mean monthly Central England Temperature (CET) records for the period 1848-2010 (Parker *et al*. 1992) were obtained from the UK Meteorological Office http://hadobs.metoffice.com/hadcet/cetml1659on.dat. This is the only complete climate record available for the years during which the museum records and NBN field data were collected. Data for Central England are drawn from a triangular area bordered by Bristol, Preston and London (Parker, Legg & Folland 1992).

Three-month mean temperature data was calculated for each year over successive periods (December (the prior year) -February; February-April; March- May. The 4-month mean for the period February-May was also calculated for each year.

Mean monthly England and Wales's precipitation figures for the period 1820-2010 were obtained from the Met Office website:

http://www.metoffice.gov.uk/research/hadleycentre/CR_data/Monthly/HadEWP_act.txt

Mean seasonal precipitation figures were calculated for spring (March-May) of each year. This was the only available historical record of precipitation to cover the whole of England and was considered the most appropriate dataset. Limited regional precipitation figures were also available but the application of such data would have been limited.

4.2.5 Geographical effects

Decimal latitude and longitude of vice county centroids were converted from their XY values. The collection site of each specimen was geo-referenced with the aid of the Ordnance Survey UK Grid Reference Finder http://gridreferencefinder.com/ and assigned to its vice county using the Digitised Watsonian Vice County Boundary data accessed via Herbaria United and the Botanical Society of the British Isles http://herbariaunited.org/gridrefVC/

4.2.6 Analysis

Correlation and regression analyses were carried out with SPSS 18 (IBM Corp., Armonk, NY, USA). Slopes and intercepts of regressions were compared via analysis of covariance (ANCOVA) (Zar 1984) using Graphpad Prism 5 (Graphpad Software Inc., La Jolla, CA, USA).

(a)
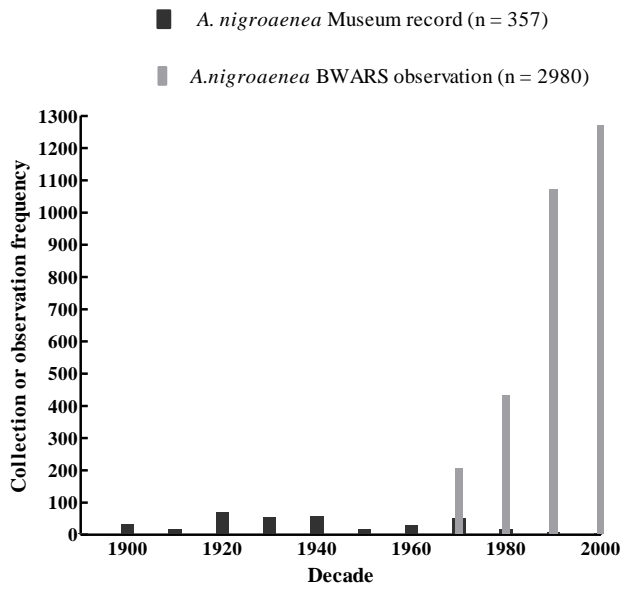
(b)
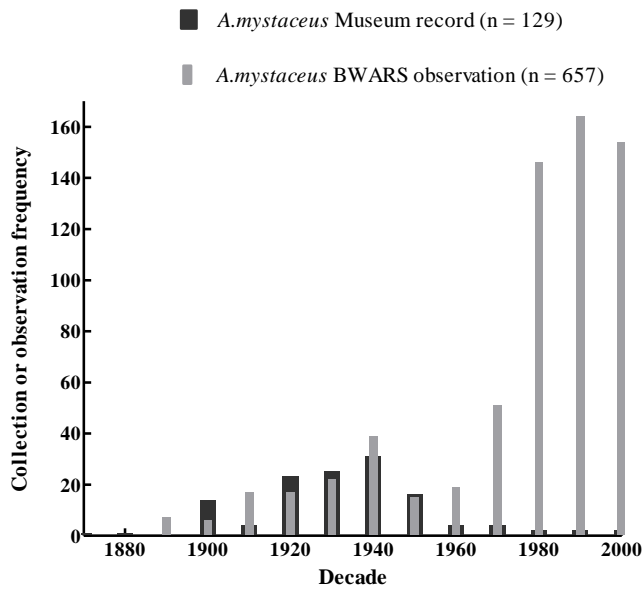
Figure 4-3. Summary of museum collection and BWARS field observation data over time for; (a) *Andrena nigroaenea* [326 (11%) of BWARS data were pre 1975. These were not included in the analysis as there was adequate data post 1975], and; (b) *Argogorytes mystaceus*.

4.2.6.1 Ophrys sphegodes

Herbarium and field observation records were expressed as days from 1 March. Both data sets had previously been examined for correlations with mean Central England temperature (CET) data in the months prior to peak flowering period (Chapter 2, sections *2.3.1* and *2.3.2* provide full details). Since linear regressions of flowering time on mean March to May temperature were statistically alike for herbarium data (1848-1958) and more recent field data (1975-2006), (Chapter 2, section *2.3.3* provides full analysis), these two sets of data were combined to generate a single dataset spanning the 159-year period 1848-2006 (Table 4-1).

4.2.6.2 Andrena nigroaenea

Museum records and BWARS observational data (expressed as days from 1 March) were both examined for correlations with mean CET data in the months prior to flight period, and significant temperature means were used in multiple regression analysis (Table 4-2).

4.2.6.3 Ophrys insectifera

Herbarium records (expressed as days from 1 March) were examined for correlations with mean CET data in the months prior to peak flowering period, and significant temperature means were used in multiple regression analysis (Table 4-1).

4.2.6.4 Argogorytes mystaceus

Museum records and BWARS observational data (expressed as days from 1 March) were both examined for correlations with mean CET data in the months prior to flight period, and significant temperature means were used in multiple regression analysis (Table 4-3).

4.3 Results

4.2.3 Correlation and multiple regression analysis

4.3.1.1 Ophrys sphegodes

Stepwise (forward) multiple regression analysis for individual month's temperature confirmed the importance of mean temperatures of March and May (Table 4-1).

Mean temperature for the 3 months March-May gave the strongest correlation with peak flowering for the combined herbarium and field dataset (Table 4-1), and this was selected as the most appropriate predictor variable.

4.3.1.2. Andrena nigroaenea

For BWARS data the highest correlation was with average temperature for the 3 months February-April but improvement over the correlations with mean temperature for the 3 months March-May, and the 4 month average for February-May, was marginal (Table 4-2). Similarly for museum records, combining male and female data, the highest correlation was with average temperature for the 4 months February-May but improvement over the correlations with mean temperature for the 3 months February-April, and March-May, was minor (Table 4-2).

Stepwise (forward) multiple regression analysis for individual month's temperature did not provide a common model. The inclusion of mean spring precipitation (March-May), whilst significantly correlated with flight time, did not significantly improve the fit in any of the regression models.

Mean temperature for the 3 months March-May was adopted as the most appropriate predictor variable for comparison of flight phenology of *Andrena nigroaenea* and flowering phenology of *Ophrys sphegodes*; this predictor generated the most significant correlation for combined herbarium and field data for *O. sphegodes* and, as a predictor for *A. nigroaenea*, was only marginally less favourable than the best fit for field observation and for museum data.

By examining the museum dataset it was possible to differentiate the flight phenology of male and female bees. Male flight phenology was most significantly correlated with

mean temperature for the 3-month period February to April, whilst female flight phenology was most highly correlated with mean temperature over the 3-month period March to May (Table 4-2). To investigate the flight phenologies of male and female bees in relation to spring temperature cues, mean February-April temperature was selected as the predictor for males, and March-May mean temperature for females.

4.3.1.3 Ophrys insectifera

The highest correlation was with average temperature for the 4-month period February-May but there were also significant correlations with mean temperature over the 3-month periods December-February, February-April, and March-May (Table 4-1).

4.3.1.4 Argogorytes mystaceus

For field (BWARS) observation data, the average for the 4 months February-May gave the highest correlation, but the improvement over the correlation with mean temperature for the 3 months March-May was small (Table 4-3). For museum data the highest correlation was with mean temperature for the 3-month period March-May, although improvement over mean temperature for the 4-month period February-May was small (Table 4-3). Mean temperature for the 3 months March-May was adopted as the most appropriate predictor variable for comparison of flight phenology of *Argogorytes mystaceus* and flowering phenology of *Ophrys insectifera*.

Stepwise (forward) multiple regression analysis for individual month's temperature did not provide a universal model suitable for all datasets. The inclusion of spring precipitation (March-May), whilst significantly correlated with flight time for museum data, did not significantly improve the fit in regression modelling.

By examining the museum dataset it was possible to differentiate the flight phenologies of male and female wasps. Female flight phenology was most significantly correlated with average temperature for the 3 month period March-May (Table 4-3), whilst male flight phenology was most highly correlated with mean temperature over the 4-month period February to May (Table 4-3).

Table 4-1. Correlations between flowering date, temperature, precipitation, latitude and longitude for; (a) combined herbarium and field records of *Ophrys sphegodes* and; (b) the herbarium records of *Ophrys insectifera*. A negative correlation indicates that a higher mean temperature or precipitation is associated with earlier flowering.

Period of temperature mean	*Ophrys sphegodes* Herbarium plus field data (1848-2006) $n = 102$	*Ophrys insectifera* Herbarium data (1835-1970) $n = 114$
June	-0.146	-0.225** [2]
May	-0.308** [2]	-0.108
April	-0.197	-0.280** [3]
March	-0.430** [1]	-0.255**
February	-0.215*	-0.307** [1]
January	-0.114	0.056
3-month means:		
March-May	-0.477**	-0.330**
February-April	-0.381**	-0.385**
December [Y]-February	-0.159	-0.228**
4-month mean:		
February - May	-0.430**	-0.392**
March-May precipitation mean:	0.158	-0.015
Decimal latitude of vice county centroid	0.377**	-0.120
Decimal longitude of vice county centroid	0.400**	-0.039
Max. r^2 for all temperature factors in stepwise forward multiple regression	0.232	0.207
r^2 using chosen 3-month mean: (March- May)	0.228	0.109

Significance: ** P < 0.01; *P < 0.05 (one tailed) , **Symbols:** [Y] = prior year; [1] [2] [3] = order in stepwise (forward) regression

Table 4-2. Correlations between flight date, temperature, precipitation, latitude and longitude for the BWARS and museum records of *Andrena nigroaenea*.

Period of temperature mean	BWARS data (1975-2009) Combined sex n = 2,980	Museum data (1893 – 2007) Combined sex n = 357	Males n = 208	Females n = 149
June	0.030 [4]	-0.006	0.145*	-0.168*
May	-0.125** [5]	-0.077 [5]	0.039	-0.204** [3]
April	-0.147** [2]	-0.185** [3]	-0.135*	-0.254** [1]
March	-0.159** [3]	-0.253** [2]	-0.272** [2]	-0.245** [2]
February	-0.186** [1]	-0.266** [1]	-0.354** [1]	-0.171*
January	-0.097**	-0.178** [4]	-0.243** [3]	-0.113
3-month means:				
March- May	-0.210**	-0.314**	-0.237**	-0.408**
February-April	-0.216**	-0.349**	-0.396**	-0.309**
December[Y-] February	-0.144**	-0.231**	-0.306**	-0.154*
4-month mean:				
February- May	-0.215**	-0.362**	-0.371**	-0.362**
March-May precipitation mean:	0.103**	0.158**	0.196**	0.105
Decimal latitude of vice county centroid	-0.045**	0.193**	0.141*	0.249**
Decimal longitude of vice county centroid	-0.049**	0.119*	0.127*	0.119
Max. r^2 for all temperature factors in stepwise forward multiple regression	0.054	0.153	0.169	0.167
r^2 using chosen 3-month mean:	0.044	0.099	0.157	0.167
a, b, d (March-May)				
c (February -April)				

Significance: ** P < 0.01; *P < 0.05 (one tailed) , **Symbols:** [Y] = prior year; [1] [2] [3] [4] [5] = order in stepwise (forward) regression

4.3.2 The Ophrys sphegodes-Andrena nigroaenea *pollination system*

There was marked similarity between the phenological responses to spring (March-May) temperature of *Ophrys sphegodes* and its pollinator, *Andrena nigroaenea* (Fig. 4-4). A 1°C rise in mean spring temperature in Central England was associated with an advance in flowering of 6.4 days of the orchid, compared with an advance in mean flying time of 7.4 days of the bee, according to the BWARS records; the slopes and intercepts of the regression models for *O. sphegodes* and *A. nigroaenea* were not significantly different (slopes, $F_{1,3078} = 0.1075$, $P = 0.743$; intercepts, $F_{1,3079} = 0.7069$, $P = 0.401$). The BWARS data were very variable, spring temperature only accounting for 4.4 % of the variation in flight date, compared with 23% of the variation in flowering date of the orchid.

The regression of flight date on temperature derived from all museum specimens collected between 1893-2007 ($y = 165.3 - 11.45x$, $r^2 = 0.10$, $P < 0.001$, $n = 357$) was also highly significant and yielded similar results to the more recent BWARS field records: a 1°C increase in mean temperature between March and May was associated with an advance in flight time of 11.5 days and temperature accounted for 10% of the variation in flight time. Despite the difference in collection period between herbarium specimens, mostly collected during the early 1900s, and the modern field observations, the differences between the slopes of the regression models derived from museum specimens and the field were not significant ($F_{1,3333} = 3.2671$, $P = 0.071$). The intercepts were significantly different ($F_{1,3079} = 33.74$, P < 0.0001). As it was possible to distinguish the sexes of *Andrena* in the museum collections, the separate phenological response of males and females were examined (Fig. 4-5). Average flight date (days from 1 March) of males was 68.8 ± 1.5, whilst average flight date of females was 72.8 ± 2.1 (Table 4-4). On average, males, the potential pollinators, flew 4.05 ± 2.57 days earlier than females, although this difference was not significant. Their flying date was also more highly correlated with mean temperature between February and April than that between March and May (Table 4-2), unlike females, which were more highly correlated with mean temperature in the later period (Table 4-2). For males a 1°C increase in mean temperature between February-April was associated with an advance in flying date of 9.2 days and the regression accounted for

15.7% of the variation in flight (Fig. 4-5). For females a 1°C increase in mean temperature between March and May was associated with an advance in flying date of 15.6 days, accounting for 16.7% of the variation in flying date (Fig. 4-5).

Table 4-3. Correlations between flight date, temperature, precipitation, latitude and longitude for the BWARS and museum records of *Argogorytes mystaceus*.

Period of temperature mean	BWARS data (1891-2010) Combined sex N = 657 [1]	Museum data (1879-2007) Combined sex N = 129	Males N = 43	Females N = 86
June	-0.125** [3]	-0.162*	-0.084	-0.234*
May	-0.175**	-0.139	-0.055	-0.239* [1]
April	-0.097**	-0.207** [2]	-0.331* [1]	-0.172
March	-0.238** [1]	-0.225** [1]	-0.155	-0.201*
February	-0.218** [2]	-0.170*	-0.152	-0.209* [2]
January	-0.129**	0.015	-0.020	-0.013
3-month means:				
March- May	-0.256**	-0.324**	-0.299*	-0.333**
February- April	-0.250**	-0.272**	-0.284*	-0.271**
December[Y] -February	-0.192**	-0.032	-0.076	-0.086
4- month mean:				
February-May	-0.265**	-0.308**	-0.301*	-0.327**
March-May precipitation mean:	-0.024	0.230**	0.150	0.218*
Decimal latitude of vice county centroid	0.248**	0.119	0.389**	0.047
Decimal longitude of vice county centroid	-0.087*	-0.281**	-0.105	-0.250*
Max. r^2 for all temperature factors in stepwise forward multiple regression	0.083	0.091	0.109	0.113
r^2 using chosen 3-month mean: (March-May)	0.066	0.105	0.09	0.111

Significance: ** $P < 0.01$; *$P < 0.05$ (one tailed); [1] excluding 8 outliers ≥ 164 days from 1 March, **Symbols:** [Y] = prior year; [1] [2] [3] [4] [5] = order in stepwise (forward) regression
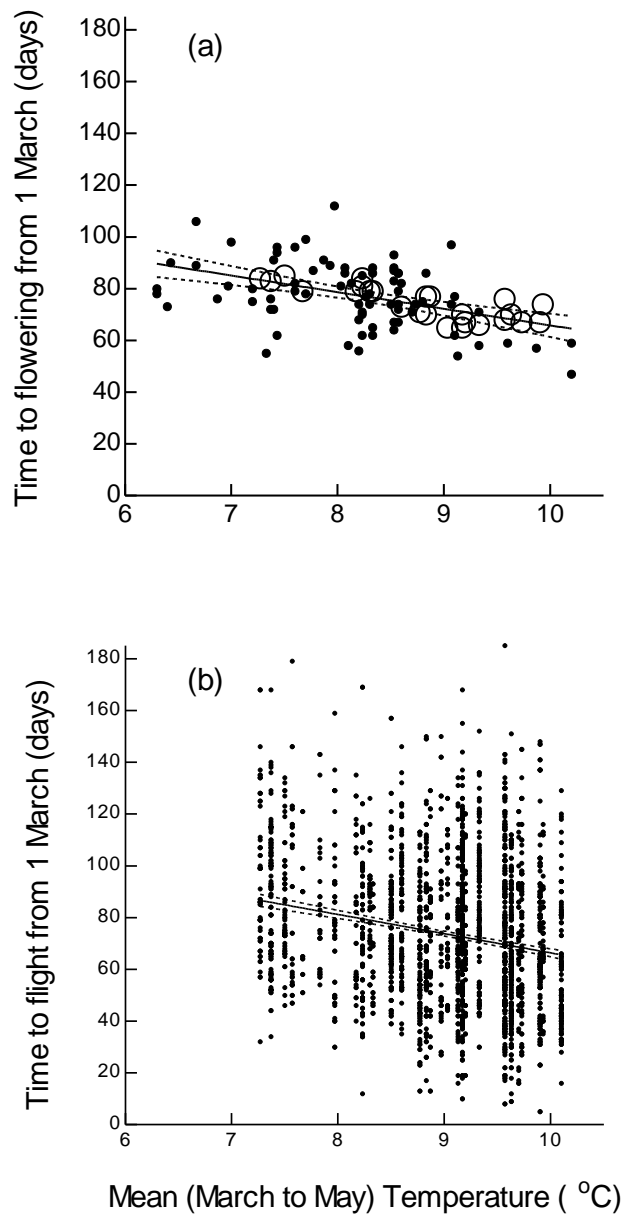
Figure 4-4. Relationship between (a) flowering date of *Ophrys sphegodes* (days after 1 March) and mean spring (March-May) temperature for combined herbarium and field data, 1848-2006 (y = 130.0 – 6.423x, r^2 = 0.23, P < 0.0001, n = 102). Field records, ○; herbarium specimens, ●., and (b) flight date of *Andrena nigroaenea* (days after 1 March) and mean spring (March-May) temperature from BWARS data, 1975-2009 (y =140.6 – 7.419x, r^2 = 0.044, P < 0.0001, n = 2,980).

Table 4-4. Estimates of mean flight times of *Andrena nigroaenea* and *Argogorytes mystaceus* and; mean flowering times of *Ophrys sphegodes* and *O.insectifera* (days from 1 March), derived from field (BWARS) records, museum and herbarium specimens.

Data source	Days from 1 March	
	Mean	S.E ±
A. nigroaenea field (BWARS; *n* = 2980)	73.8	0.5
A. nigroaenea museum-all (*n* = 357)	70.5	1.3
A. nigroaenea museum-male (*n* = 208)	68.8	1.5
A. nigroaenea museum-female (*n* = 149)	72.8	2.1
O. sphegodes herbarium plus field (*n* = 102)	76.8	1.2
A. mystaceus field (BWARS; *n* = 657)	108.0	0.7
A. mystaceus museum-all (*n* = 129)	107.3	1.6
A. mystaceus museum-male (*n* = 43)	95.1	2.0
A. mystaceus museum-female (*n* = 86)	113.5	1.8
O. insectifera herbarium (*n* = 114)	99.9	1.0
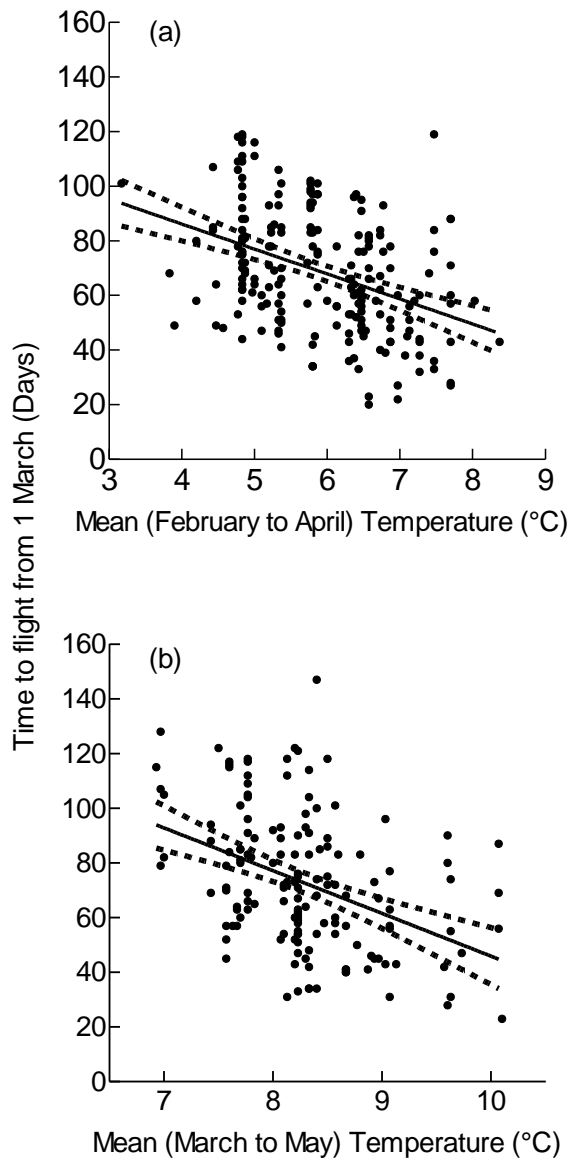
Figure 4-5. Relationship between flight date of *Andrena nigroaenea* (days after 1 March) and mean spring temperature from museum specimens. (a) males v. mean February-April temperature, 1893-2004 ($y = 122.8 - 9.168x$, $r^2 = 0.157$, $P < 0.0001$, $n = 208$); (b) females v. mean March-May temperature, 1900-2007 ($y = 202.3 - 15.64x$, $r^2 = 0.167$, $P < 0.0001$, $n = 149$).

4.3.3 The Ophrys insectifera-Argogorytes mystaceus *pollination system*

The data for flowering time in *Ophrys insectifera* derived from the herbarium records collected between 1835 and 1970 were highly correlated with mean spring temperature. A 1°C increase in mean temperature between March and May was associated with an advance in flowering time of 4.4 days (Fig. 4-6a) and the regression accounted for 10.9 % of the variation in flowering time.

There were similar responses in the flying time of *Argogorytes mystaceus* to spring temperature in both the BWARS field data and that from museum specimens, notwithstanding the different eras over which these sets of data were collected. For field observations, a 1°C increase in mean spring temperature (March-May) was associated with an advance in flight date of 5.8 days, with the regression accounting for 6.7% of the variation in flight date (Fig. 4-6b), compared with an advance of 7.4 days and a regression accounting for 10.5% of the variation based on museum records (Fig. 4-6c). The regression models derived from field observation and museum collections were statistically indistinguishable (slopes, $F_{1,782}$ = 0.533, P = 0.466; intercepts $F_{1,783}$ = 1.5908, P = 0.208). Furthermore, the slopes of the regressions of flowering date for *O. insectifera* and flight date for *A. mystaceus* on spring (March-May) temperature derived from field observations, were not significantly different ($F_{1,767}$ = 0.4389, P = 0.508). However, the difference between the intercepts was significant ($F_{1,768}$ = 41.04, P < 0.001) presumably reflecting the geographical variation in collection sites. In this case there was no difference in the phenological response of males and females to mean (March-May) temperature ($F_{1,125}$ = 0.4373, P = 0.51) indicating a common phenological response for flying date in both sexes to mean spring temperature between males and females. The difference between the intercepts was significant ($F_{1,126}$ = 40.6388, P < 0.001), and was reflected in the difference in mean flight date between the sexes of 18.44 ±2.67 days; mean date (days from 1 March) of male flight was 95.1 ± 2.0, compared to mean flight of females of 113.5 ± 1.8 (Table 4-4).
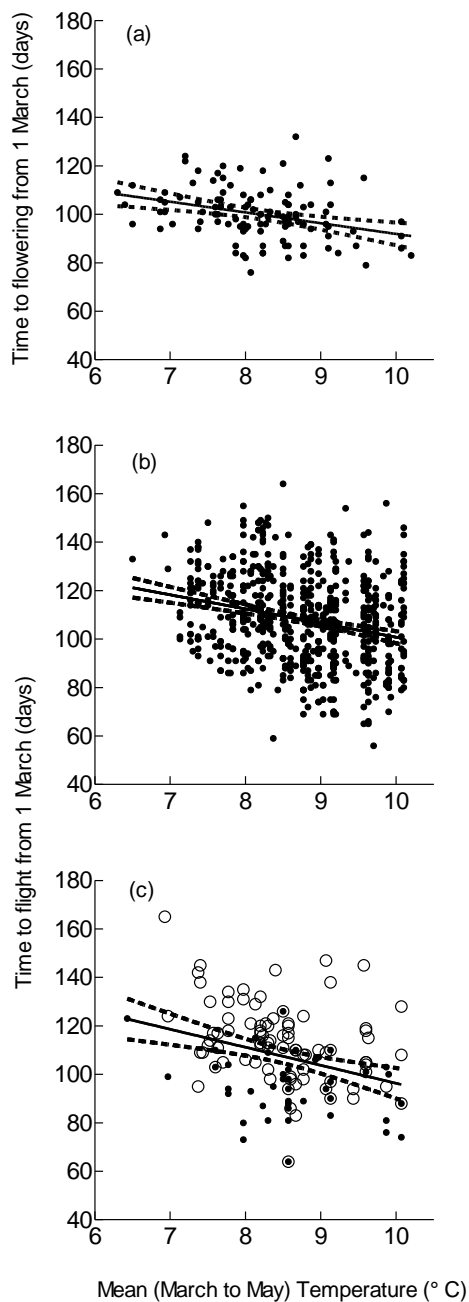
Figure 4-6. (a) Relationship between flowering date of *O. insectifera* (days after 1 March) and mean spring (March – May) temperature from herbarium data, 1835-1970 ($y = 136.3 - 4.434x$, $r^2 = 0.109$, $P < 0.001$, $n = 114$); (b): Relationship between flight date of *A. mystaceus* and mean spring temperature for BWARS data, 1891-2010 ($y =159.1 - 5.833x$, $r^2 = 0.066$, $P < 0.0001$, $n = 657$); (c) Relationship between flight date of *A. mystaceus* and mean spring temperature from museum data; female, ○; male, ●; 1879-2006 ($y =170.6 - 7.432x$, $r^2 = 0.105$, $P < 0.001$, $n = 129$).

4.3.4 Correspondence between flying date of Andrena nigroaenea *and flowering date of* Ophrys sphegodes.

As flowering of *O. sphegodes* and flight activity of males of *A. nigroaenea* were best correlated with mean temperature over different periods of the spring, direct comparison of the phenological responses of male and female bees and the orchid was difficult. Museum and herbarium records were relatively sparsely distributed over time so that there were few years containing data for all three phenological events; orchid flowering, and flight of male and female bees. Consequently flowering time was predicted for each year using the regression model derived from the combined herbarium and field records for *O. sphegodes* and this was compared, using the museum records, with actual flying dates of male and female bees. In the 62 years in which males were collected, the proportion of years in which the mean collection date of male bees preceded the predicted flowering date increased with increasing mean March-May temperature between 7.1-9.9°C (Fig. 4-7a). A single year in which mean spring temperature was less than 7°C and two years in which it was greater than 10°C have been excluded because of the scarcity of data in these classes.

In the 43 years in which both males and females were collected, the proportion of females in all bees collected on or before predicted flowering date increased with increasing mean March-May temperature between 7.4-9.6°C (Fig. 4-7b). A single year in which mean spring temperature was less than 6°C and one year in which it was greater than 10°C have been excluded because of the scarcity of data

In the 38 years in which males or females were collected before predicted flowering date the fraction of years in which females were collected showed an even more striking increase with temperature (Fig. 4-7c).

Similar comparisons between the phenology of *O.insectifera* and males and females of *A. mystaceus* were not possible because of the limited number of years in which males and females were in flight.
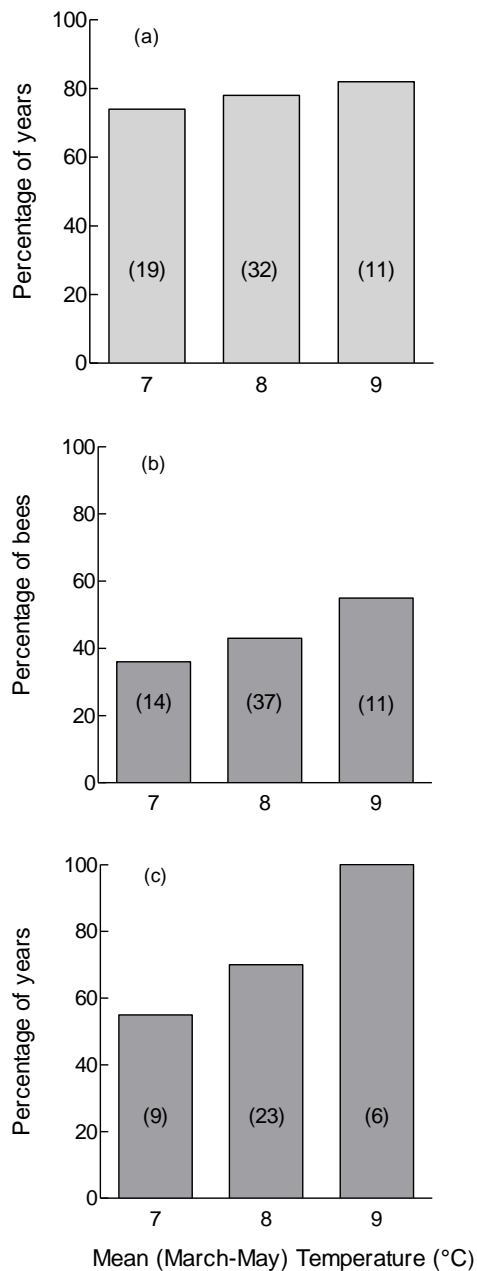
Figure 4-7. Effects of mean spring (March-May) temperature on flight date of *A. nigroeanea* relative to predicted flowering of *O. sphegodes*: (a) proportion of years in which the mean collection date of males was on or before predicted flowering date, based on 62 years in which males were collected; (b) proportion of females in collections on or before predicted flowering date in 43 years in which both males and females were collected; (c) proportion of years in which females flew on or before predicted flowering date in 38 years in which either males or females were collected on or before predicted flowering date. Symbols: (*n*) = number of years of observation (a, c); or number of observations (b).

4.4 Discussion

The phenological response of *Ophrys sphegodes* to spring temperature detected in herbarium specimens collected over a period of 111 years has previously been validated by its close agreement with that derived from direct recordings of mean flowering date over a period of 32 years (Robbirt *et al.* 2011) (Chapter 2). Combining herbarium and field records into a single dataset spanning a period of 159 years provided a robust model for *O. sphegodes* with an estimated advance in peak flowering per 1°C rise in mean spring temperature of 6.4 days.

It is likely that the similar response of *O. insectifera* seen in the herbarium material over a 136-year period is equally valid: the estimated advance in peak flowering date per 1°C rise in mean spring temperature of 4.4 days was identical with the mean advance in first flowering date per 1°C rise in mean spring temperature averaged across 243 species at a single locality in Oxfordshire, England (Fitter *et al.* 1995). Both orchid species fall within the range of 2 to 10 days advance in mean flowering seen for 24 species across the UK per 1°C rise in temperature (Sparks, Jeffree & Jeffree 2000).

The extensive field data provided by members of BWARS, mostly observed between 1970 and 2010, showed a significant trend toward advancement in flying date in relation to mean spring (March-May) temperature: 7.4 days per 1°C for *Andrena nigroaenea* and 5.8 days for *Argogorytes mystaceus*. Whilst other meteorological factors such as sunshine hours and precipitation undoubtedly influence the collection and observation of insects, the effect of temperature gave a clearly discernible phenological signal for both species in this study. There are few prior studies for Hymenoptera, but Bartomeus *et al*. (2011) recently reported an average advance of 3.6 days per 1°C rise in April temperature for 10 common bee species in North America. For the honey bee *Apis mellifera*, mean first appearance date in the Iberian Peninsula has advanced significantly with rising mean spring February-April temperature, since 1970 (Gordo & Sanz 2006). Young of most solitary bees, including *A. nigroaenea* overwinter as pupae in underground nests, emerging as young adults in the spring (Baldock 2008), and therefore emergence and flight date would be expected to be sensitive to temperature.

Comparison of these field-based observations with data derived from museum specimens over a longer time span provided validation for using museum records: neither the estimated advance in flight date derived from museum specimens of 11.5 days for *A. nigroaenea*, nor 7.4 days for *A. mystaceus*, were significantly different to the observation data. This was the case despite the two sets of data being dissimilar in geographic range, contrasting in length and of separate time periods.

Museum data for both *A. nigroaenea* and *A. mystaceus* was marginally more sensitive to spring temperature than BWARS field data, although for neither species were the differences significant. The ability to determine the sex of museum specimens was important since only the males serve as pollinators, whilst the females are stronger competitors than the flowers in attracting the copulatory attentions of the males. In the museum records for *A. nigroaenea* the earlier average flight date of males over females of *c.*4 days was as expected (Eickwort & Ginsberg 1980). Average flight date of males was more sensitive to temperature earlier in the year than for females: male flight date was significantly associated with temperatures from January whilst females were associated with mean monthly temperatures from February onwards. Even so, females responded more strongly (15.6 days per 1°C) to mean March-May temperature than the males (9.2 days per 1°C) to mean February-April temperatures; the seasonal periods of highest significance for females and males respectively.

The museum data for the wasp *A. mystaceus* gave an earlier average flight date for females over males of *c.*18 days, which was considerably greater than anticipated. The museum data for *A. mystaceus* was derived from a much smaller set of records than that for *A. nigroaenea*, and given the low signal to noise ratio in both datasets, geographical range across which the insects were collected, and relatively long flight period of females, the dataset for *A. mystaceus* may have been too small to generate a firm phenological signal for each sex separately. In contrast to the findings for *A. nigroaenea*, there was no significant difference between phenological responses of flying time in the males and females of the wasp.

The pollination systems of *O. sphegodes* and *O. insectifera* are highly specific; flowers achieve cross pollination by attracting newly emerged males of *A. nigroaenea* (Ayasse

et al. 2000; Schiestl *et al.* 2000; Paxton & Tengö 2001; Mant, Peakall & Schiestl 2005) and *A. mystaceus* (Borg-Karlson 1990; van der Cingel 1995) respectively, using chemical and visual cues. The phenology of the male bees is therefore important both in relation to flowering time and to the phenology of the female bees, representing a rival attraction for the attention of males (Nilsson 1992). The findings of this study demonstrate the close proximity understood between flowering and flight activity. These results also support the scenario of competition between orchid flowers and females; mean date of female flight (73 days from 1 March) and orchid flowering (77 days from 1 March) are not significantly different, whilst male flight (69 days from 1 March) is significantly earlier than flowering.

Potentially disruptive effects on species interactions may occur if there are significant differences in responses of individual species to climate warming (Memmot *et al.* 2007; Willis *et al.* 2008; Thackeray *et al.* 2010; Bartomeus *et al.* 2011; Rafferty & Ives 2011) especially for highly specific pollination systems as seen in *Ophrys*. For the *O. insectifera - A. mystaceus* pollination model there was no indication that asynchrony may occur with climate warming. In contrast, comparing the phenological responses of *A. nigroaenea* and *O. sphegodes* to mean spring temperature suggests that there is potential for disruption of synchrony between plant and pollinator with further rises in spring temperature. The probability of both males and females flying earlier than predicted peak flowering of *O. sphegodes* rose with increasing mean spring temperature, suggesting that flight of males will advance faster than the advance in flowering. Therefore the availability of male bees as pollinators, already limited (Ayasse *et al.* 2000), is likely to decline with climate warming.

Limited knowledge has been accumulated toward understanding how climate warming affects plant-pollinator interactions (Hegland *et al.* 2009) and there are few prior studies. Of these, Gordo & Sanz (2005) found advancing spring phenologies of the honey bee (*Apis mellifera*) and the small white butterfly (*Pieris rapae*) relative to host plants, whilst Kudo *et al*. (2004) found insect phenology to be more muted in response to temperature than flowering phenology of the host plant. A recent study suggested that on average bee flight phenology is advancing in line with flowering of host plants (Bartomeus *et al.* 2011). It is apparent from the few studies to date that further research

of closely associated phenological relationships would be valuable. Even so, predictions of future phenologies beyond modest temperature ranges should be cautious: linear relationships between flowering and flight phenologies and measures of temperature seen in this study and others (Sparks, Jeffree & Jeffree 2000; Stefanescu, Penuelas & Filella 2003; Bartomeus *et al.* 2011) , may be expected to deviate eventually due to the influence of other constraining factors (Hegland *et al.* 2009), such as photoperiod requirements.

In conclusion, this research uses long-term empirical data derived from museum and herbarium specimens to investigate the relative phenologies of two sexually deceptive orchids, *Ophrys sphegodes* and *O. insectifera* and their respective pollinators; the aculeate Hymenoptera *Andrena nigroaenea* and *Argogorytes mystaceus*. The value of museum data as proxy for field study is affirmed by evaluation of results derived independently from historical museum specimens and from more recent field-based observational records, for both pollinator species. Results of this research suggest that, whilst the potential for asynchrony in ecological interactions due to climate change are complex and difficult to predict, there is evident potential for loss of synchrony in tightly co-evolved systems, as described here between the sexually deceptive orchid *O. sphegodes* and its Hymenoptera pollinator *A. nigroaenea*.

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Chapter 5

Orchid-butterfly relationships: an evaluation of the potential for plant-pollinator asynchrony under climate warming

Summary

1. This study uses long-term data from herbaria and museum collections to examine the potential for asynchrony under climate warming, between selected British orchids and the univoltine Lepidoptera that pollinate them.

2. Herbarium data for *Anacamptis pyramidalis* recorded in the UK between 1830 and 1980 were compared to museum data of *Euclidia glyphica* recorded between 1887 and 1972, and to field data for *E. glyphica* recorded between 1956 and 2010. Phenological responses of flowering and of flight to variation in mean temperatures for the three-month period March-May were statistically alike. A 1°C rise in mean (March-May) temperature was associated with an advance in flowering of *c*. 7.1 days. Using combined museum and field data of *E. glyphica*, a 1°C rise in mean (March-May) temperature was associated with an advance in flight of 5.8 days. Similar results were found in responses to mean temperature for the three-month period April to June. A 1°C rise in mean (April-June) temperature was associated with an advance in flowering of *c*. 11.3 days and an advance in flight of 7.8 days.

3. Museum data of *Melanargia galathea* collected in the UK between 1890 and 1998 were compared to herbarium data of *Anacamptis pyramidalis* recorded between 1830 and 1980. Phenological responses of flowering and of flight to variation in mean temperatures for the 3-month period June to August were statistically different. A 1°C rise in mean (June-August) temperature was associated with an advance in flowering of *c*. 8.5 days and an advance in flight of 4.3 days.

4. Museum data of *Deilephila elpenor* recorded in the UK between 1893 and 1992 were compared to field data recorded at Wicken Fen, Cambridgeshire between 1899 and 2009, and to herbarium data of *Platanthera bifolia* recorded between 1852 and 1965 and to *Platanthera chlorantha* recorded in the UK between 1835 and 1962. Phenological responses of flowering of *P. bifolia* and of flight of *D. elpenor* to variation in mean temperatures for the 3-month period March to May were statistically

indistinguishable. A 1°C rise in mean (March-May) temperature was associated with an advance in flowering of 5.8 days and an advance in flight of *c*. 5.3 days. Similarly phenological response for flowering of *Platanthera chlorantha* and flight of *D. elpenor* were analogous. For *P. chlorantha* a 1°C rise in mean (March-May) temperature was associated with an advance in flowering of *c*. 4.7 days.

5. These results suggest that under current rates of climate warming in the UK, flight phenologies of Lepidopteran pollinators may remain in synchrony with flowering phenologies of their associated early summer-flowering orchids. Even so, earlier flowering of the mid-summer-flowering orchid *Anacamptis pyramidalis* suggest that there is the potential for asynchrony between its flowering phenology and the flight phenology of what is a proxy for mid-summer pollinators (*Melanargia galathea*).

5.1 Introduction

Timing of seasonal events is closely linked to temperature. Sensitivity to climate may not only differ between trophic levels (Voigt *et al.* 2003), but species may respond to different climatic cues (Doi, Gordo & Katano 2008) leading to a breakdown in synchronicity (McCarty 2001; Stenseth & Mysterud 2002; Stenseth *et al.* 2002; Dixon 2003). Insects often exhibit complex life histories, evolved to stay in synchrony with key seasonal events (Hodgson *et al.* 2011), and their phenologies respond to specific cues, such as temperature and day-length (Bale *et al.* 2002). It is recognised that a detailed knowledge of phenological relationships at species level are needed toward an understanding of the community consequences of climate-driven phenological shifts (Nakazawa & Doi 2012).

Insects are poikilothermic and temperature is the dominant abiotic factor in insect development and activity (Bale *et al.* 2002; O'Neill *et al.* 2012). Photoperiod is also a significant factor and its interaction with temperature is not well understood (Bale *et al.* 2002). Forecasts of insect phenology are sometimes based on preceding temperatures, such as accumulated temperature and achievement of accumulated threshold values of 'day-degrees'(Dewar & Watt 1992; Watt & McFarlane 2002) and similarly, growing degree days above a threshold temperature (Hodgson *et al.* 2011). It is interesting that some studies have shown differences between insect development rates in the field and in the laboratory (Fielding *et al.* 1999; Bale *et al.* 2002) and Lepidoptera larvae are reported to be able to adapt their behaviour in order to influence their thermal environment (Bryant, Thomas & Bale 2000; Bale *et al.* 2002).

Breakdown in synchronicities between plants and insects may be expected where there are differences in sensitivities to temperature change above thermal development thresholds, or chilling requirements, or where photoperiod requirements exist (Watt & McFarlane 2002). Mismatch of phenophases associated with climate warming have been reported for primary producers and associated herbivores with negative impact on population abundance (Watt & McFarlane 1991; Watt & Woiwod 1999; Visser & Holleman 2001; Winder & Schindler 2004). Phenological changes have been shown to affect synchrony of larvae and host plant (Watt & McFarlane 1991; Watt & Woiwod 1999) with negative consequences for insect abundance. Conversely climate warming

may positively influence abundance if additional broods are achieved in warm years (Woiwod 1997) or when distribution range is shifted or expanded (Pollard, Moss & Yates 1995; Parmesan 1996).

Changes in phenology associated with climate warming have been demonstrated for plant species (Fitter *et al.* 1995; Menzel *et al.* 2006) including temperate orchids (Robbirt *et al.* 2011; Molnár *et al.* 2012), and for Lepidoptera (Brakefield 1987; Sparks & Yates 1997; Stefanescu, Penuelas & Filella 2003; Dingemanse & Kalkman 2008; Hodgson *et al.* 2011; O'Neill *et al.* 2012). For Lepidoptera earlier first flight dates and longer flight periods are both associated with increased temperatures (Roy & Sparks 2000; Forister & Shapiro 2003; Westwood & Blair 2010).

Due to the small number of empirical studies, limited knowledge has been accumulated toward understanding how climate warming affects plant-pollinator mutualisms (Hegland *et al.* 2009) or their demographic consequences. Given the variation in species response, some studies have reported mismatch in phenophases (Kudo *et al.* 2004) whilst others report parallel temporal responses (Bartomeus *et al.* 2011). Simulations have demonstrated the vulnerability of plant-pollinator networks to climate warming (Memmot *et al.* 2007) particularly for specialised pollinators, although there is suggestion that multi-species plant-pollinator networks may be sufficiently robust to withstand phenological adjustment arising from climate change (Hegland *et al.* 2009). As illustration of this, Petanidou *et al.* (2008) found that where fidelity is low, phenological asynchrony may provide the opportunity for fresh plant-pollinator relationships. Depending on the current level of synchrony, climate change may provide convergent forces for some species (Parmesan 2007). Nevertheless, the vulnerability of plant-pollinator relationships to phenological mismatch depends on the starting point, and species that are not currently well adapted to their habitat may be vulnerable to small changes in synchrony (Parmesan 2007). In a recent meta-analysis, however, the advancement in first flight of butterflies was 3-fold greater than first flowering of plants (Parmesan 2007).

A number of British orchid species rely on Lepidoptera for pollination. These include rewarding (*Platanthera bifolia, P. chlorantha, Gymnadenia conopsea, G. densiflora* and *G. borealis,*) and non-rewarding (*Anacamptis pyramidalis*) species. Pollinators

comprise day flying butterflies as well as day and dusk or night flying moths. Spur length or fragrance may determine the suite of pollinators for each species.

Few published data currently exists of the relative phenologies of plants and pollinators, and none is available for temperate orchid species. Long-term datasets on the phenology of invertebrates are scarce (Roy & Sparks 2000) due in part to the extensive resources required to collect such records (Woiwod 1997) and the general preference for short-term experimental data (Woiwod 1991).In the UK much of the monitoring effort has been focused on agricultural pests (Fleming & Tatchell 1995; Zhou *et al.* 1995), and whilst citizen-science databases are valuable there can be drawbacks, such as inconsistencies in observer knowledge (O'Neill *et al.* 2012).

Even the high profile and relatively well studied Lepidoptera lack extensive data to enable phenological effects to be routinely assessed (Woiwod 1997). Since 1976, however, the Butterfly Monitoring Scheme (BMS) has accumulated a substantial amount of observational data on butterflies in the UK, most of which is collated from amateur observation. Yet long-term collections held in museums such as the Natural History Museum (BM), London, represent a valuable, largely unrecognised, resource for phenological studies. The aim of this research was to utilise museum collections of Lepidoptera to evaluate the potential for asynchrony between selected orchids and their pollinators. Specific relationships examined were between *Anacamptis pyramidalis* and two of its potential pollinators *Euclidia glyphica* and *Melanargia galathea*. The relationships between *Platanthera bifolia* and *P. chlorantha* and an important pollinator of both orchid species *Deilephilor elpenor* were also examined. The specific hypotheses were that *i)* peak flight would be advanced by warmer spring and summer temperatures *ii)* the relationship between peak flight and temperature would be the same in data derived from museum collections and field based records *iii)* using museum collections flight phenology of pollinating butterfly and moth species could be critically compared to flowering phenology of associated orchids.

5.2. Methods

5.2.1 The study species

5.2.1.1 Euclidia glyphica (L.) Burnet Companion

A member of the family Noctuidae, sub-family Catocalinae, *Euclidia glyphica* is one of the few diurnal moths species native to Britain (Fig. 5-1d), and is often seen in the company of *Zygaena* spp. (Burnet moth) and *Callistege mi* (Mother Shipton), both of which are also diurnal. The wingspan of *E. glyphica* is typically 25 to 30mm (Novák 1980), and the distinct appearance of the species is marked by the orange-yellow hind-wings combined with the warm brown forewings Whilst it is common in central and southern England sightings become much more restricted in Wales, northern England, southern Scotland and Ireland, and it is scarce on the Channel Islands (Waring & Townsend 2009). Favoured habitats are open wood, meadows, pasture downland, and sometimes along roadside verges and embankments. The larvae feed during late June to August, and overwinter as pupae. Favoured larval foodplants include *Trifolium pratense* (L.) (Red Clover), *Trifolium repens* (L.) (White Clover), *Lotus corniculatus* (L.) (Common Birds-foot-trefoil), *Medicago lupulina* (L.) (Black Medick), *M. s. ssp. sativa* (Lucerne) and *Vicia cracca* (L.) (Tufted Vetch) (Waring & Townsend 2009). In Central Europe there are sometimes two broods flying between April and August (Novák 1980), but in Britain there is a single generation emerging between May and June, flying between May and July (Waring & Townsend 2009). Reported flight activity for this species is most frequent in sunshine and warm overcast weather (Waring & Townsend 2009). In Britain *E. glyphica* is a known pollinator of *Anacamptis pyramidalis* (Darwin 1877; van der Pijl & Dodson 1966).

5.2.1.2 Deilephila elpenor (L.) Elephant Hawkmoth

A member of the Sphingidae family, *Deilephila elpenor* is one of the more common European hawkmoth species (Fig. 5-1c). Although widespread in Europe it is largely absent from northernmost regions but extends eastward into Asia and Japan, and has been recorded in mountain regions at elevations up to 1500m (Novák 1980). Wingspan is typically 45 to 60 mm. Sexes have similar markings: the olive green forewing and thorax have distinct pink patterning. Associated habitats include rough grassland, hedgerows, woodland clearings, heathland and sand dunes. Larvae overwinter as pupae, usually hidden amongst floor litter. The willowherbs, *Epilobium hirsutum* L. (Great Willowherb) and *Chamerion angustifolium* L. (Rosebay Willowherb), are primary sources of food for the larvae during late summer (Novák 1980) as well as

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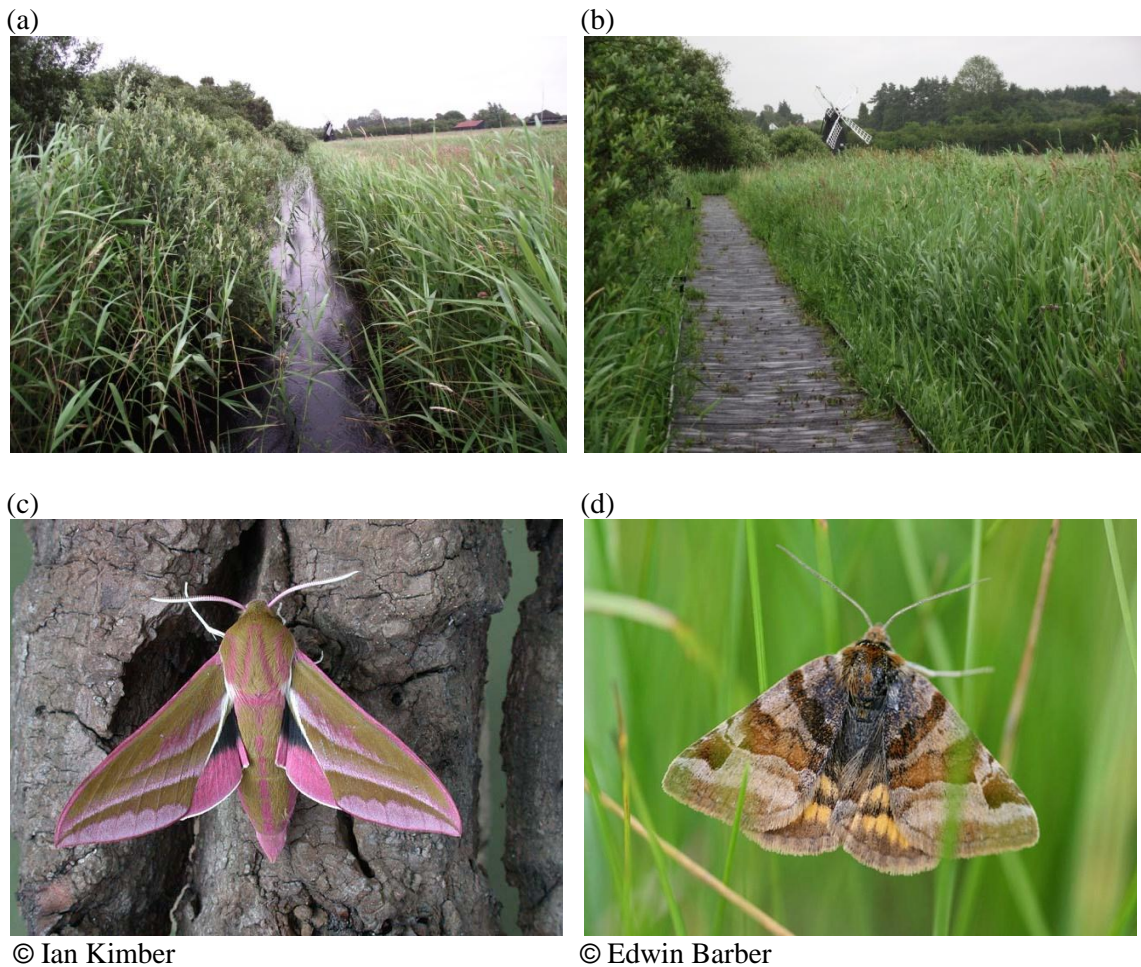
Figure 5-1. Wicken Fen (a,b) National Nature Reserve, Cambridgeshire (TL 562705) 11 July 2012, a protected fenland habitat and source of field-based observation data between 1970 and 2009 for (c) *Deilephila elpenor* (Elephant Hawkmoth); image of (d) *Euclidia glyphica* (Burnet Companion)

species of *Galium*, and other plants (Waring & Townsend 2009). It is widespread and relatively common across England, Wales and Ireland. Whilst less frequent in Scotland, it has increased its range northward in recent years (Waring & Townsend 2009). The species is single brooded in Britain with a flight period between May and early August (Waring & Townsend 2009). In Europe a second brood is sometimes seen in August and September (Novák 1980), and in recent years a small number of fresh adults have also been recorded during the late summer in southern and central England (Waring & Townsend 2009).

5.2.1.3 Melanargia galathea (L.) Marbled White

Melanargia galathea is a member of the butterfly family Satyrinea. This species is distributed across areas of central Europe, but is absent from Portugal, most of Spain, the Mediterranean islands, and Scandinavia. It is also present in northern Africa. In Britain this species is primarily confined to southern and south-western counties of England. Here it can be locally common with extensive colonies sometime comprising several hundred individuals. Scattered colonies exist in Yorkshire, south Wales and central England. However the butterfly's range has contracted greatly, thought to be due chiefly to loss of habitat. It is entirely absent from Scotland, Ireland, Isle of Man and the Channel Islands (Asher *et al.* 2001).

The species favours areas of unimproved species-rich chalk grassland which may form a relatively tall pasture, depending on grazing or cutting regimes. There is a single generation each year across its range. Adults emerge in mid June, reaching a peak in mid-July, and staying on the wing into August (Asher *et al.* 2001). Males and females can be easily distinguishes by the colour of the underside of the wing: males have dark, almost black, veining whilst females have veining of a light brown colour. The female releases her eggs in August and the emergent larvae almost immediately enter hibernation typically amongst *Festuca rubra,* a favoured food source of the larvae (Asher *et al.* 2001). Emerging in spring the instars feed at night prior to pupation, which lasts around three weeks. Adults demonstrate a marked preference for purple flowers, including Thistles (*Cirsium* spp. and *Carduus* spp.), Wild Marjoram (*Origanum vulgare*), Field Scabious (*Knautia arvensis*) and Knapweeds (*Centaurea* spp.) (Asher *et al.* 2001). Their preferred habitat of well-drained species-rich calcareous grassland is also associated with many British orchids. Sightings of *Melanargia galathea* visiting *Anacamptis pyramidalis* (Fig. 5 -2a) and *Gymnadenia conopsea* (Fig. 5-2b) indicate that this butterfly may be a pollinator species for these summer-flowering orchid species.

(a) (b)
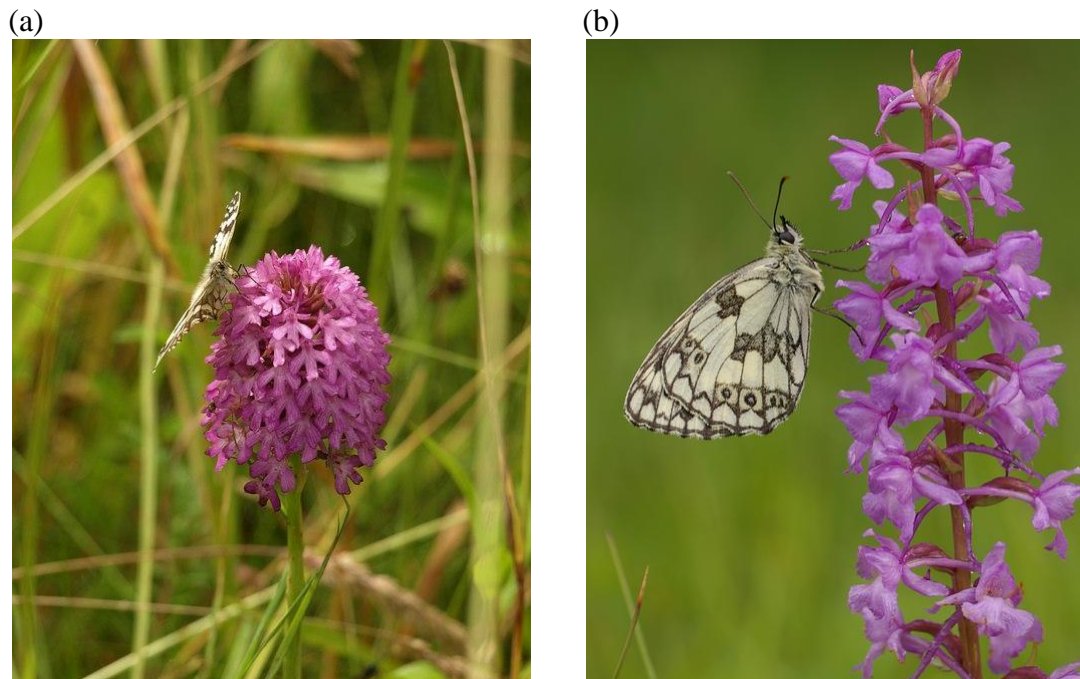
© Derek Harper

Figure 5-2. Images of (a) *Melanargia galathea* on *Anacamptis pyramidalis* on 6 July 2008, at Barton, Torbay, UK (decimal latitude 50.48925, decimal longitude -3.54877), and (b) *Melanargia galathea* on *Gymnadenia conopsea* 7 July 2009, Rosière, France.

5.2.1.4 Anacamptis pyramidalis (L.) Rich. Pyramidal orchid

Chapter 3, section *3.2.2.2.2* provides a full description of *Anacamptis pyramidalis* and an account of the pollination system.

5.2.1.5 Platanthera chlorantha (Custer) Rchb. (syn. *Gymnadenia chlorantha* (Custer) Ambrosi, *Habenaria chlorantha* (Custer) Bab., *Orchis chlorantha* Custer) Greater Butterfly Orchid

Chapter 3, section *3.2.2.3.2* provides a full description of *Platanthera chlorantha* and an account of the pollination system.

5.2.1.6 Platanthera bifolia (L.).Rich. (syn. *Gymnadenia bifolia* (L.) G Mey, *Habenaria bifolia* (L.) R. Br., *Orchis bifolia* L.) Lesser Butterfly Orchid

Chapter 3, section *3.2.2.3.3* provides a full description of *Platanthera bifolia* and an account of the pollination system.

5.2.2 Herbarium data

Herbarium specimens held at the Royal Botanic Gardens, Kew (K) and at the Natural History Museum (BM) were examined for the three orchid species in this study. Specimens spanned a collection period of 151 years between 1830 and 1980.

A general description of the treatment of the herbarium specimens can be found in Chapter 3, Section *3.2.1*.

5.2.2.1 Anacamptis pyramidalis

Chapter 3, Section *3.2.1* and Table 3-3 provides a summary of the records derived from herbarium specimens at Kew and the Natural History Museum, London. The final data set comprised 151 specimens (BM, 84 specimens) and Royal Botanic Gardens, Kew (K, 67 specimens), providing at least one data point for each of 75 years, spanning a 151-year period from 1830 to 1980 (Table 3-5).

5.2.2.2 Platanthera bifolia

Chapter 3, Section *3.2.1* and Table 3-3 provides a summary of the records derived from herbarium specimens at Kew and the Natural History Museum, London. The final data set comprised 110 specimens (BM, 69 specimens) and Royal Botanic Gardens, Kew (K, 41 specimens) providing at least one data point for each of 61 years, spanning a 114-year period from 1852 to 1965 (Table 3-5).

5.2.2.3 Platanthera chlorantha

Chapter 3, Section *3.2.1* and Table 3-3 provides a summary of the records derived from herbarium specimens at Kew and the Natural History Museum, London. The final data set comprised 109 specimens (BM, 54 specimens) and Royal Botanic Gardens, Kew (K, 55 specimens) providing at least one data point for each of 65 years, spanning a 128-year period from 1835 to 1962 (Table 3-5).

Table 5-1. Correlations between temperature, precipitation, latitude, longitude and flight date for the museum and observation data of *Euclidia glyphica*. Correlations are shown with mean temperatures for 3-monthly periods and individual months in the same year as flight (January- August).

	Euclidia glyphica		
	Museum (1887-1972) *n = 83*	NBN (1956 -2010) *n = 141*	Combined (1887-2010) *n = 224*
Period of temperature mean:			
August	-	-	-
July	-0.158	-0.040	-0.067
June	-0.262**	-0.117	-0.169**
May	-0.422**[2]	-0.260**[1]	-0.303**[1]
April	-0.232*[3]	-0.138	-0.167**
March	0.081	-0.228**	-0.097
February	0.035	-0.206**	-0.080
January	0.167	-0.155*	-0.008
3 month means:			
April - June	-0.443**	-0.269**	-0.310**
March - May	-0.245*	-0.311**	-0.261**
December [Y] -February	0.122	-0.248**	-0.068
Period of precipitation mean			
May	0.022	-0.092	-0.047
April	0.248*	0.103[2]	0.140*[2]
March	-0.038	0.063[3]	0.018[3]
February	0.063	0.030	0.045
January	-0.123	-0.062	-0.085
3 month means:			
March - May	0.116	0.058	0.080
decimal latitude	-0.058	-0.126	-0.091
decimal longitude	0.456**[1]	-0.045	0.084

Significance: **P <0.01; * *P* <0.05 (one-tailed), **Symbols:** [Y] = prior year;
[1] [2] [3] = order in stepwise (forward) regression

Table 5-2. Correlations between temperature, precipitation, latitude, longitude and flight date for the museum data for *Melanargia galathea*. Correlations are shown with mean temperatures for 3-monthly periods and individual months in the same year as flight (January- August).

| | *Melanargia galathea* | | |
	Combined (1890-1998) n = 161	Male (1890 -1998) n = 94	Female (1892-1979) n = 67
Period of temperature mean:			
August	-0.173*	-0.146	-0.170
July	(1) -0.232**	(2) -0.257**	-0.154
June	-0.140*	-0.139	-0.134
May	(3) -0.086	-0.033	-0.197
April	-0.097	-0.163	0.059
March	-0.056	-0.142	0.086
February	-0.031	-0.028	-0.033
January	-0.029	0.028	-0.096
3 month means:			
June - August	-0.248**	-0.247**	-0.207*
March - May	-0.129	-0.183*	-0.016
December ^{Y} -February	-0.006	0.020	0.001
Period of precipitation mean			
May	0.101	0.095	0.118
April	0.041	-0.025	0.166
March	(2) 0.162*	0.201*	0.059
February	0.011	-0.063	0.138
January	-0.073	0.009	-0.199
3 month means:			
March - May	0.191**	0.181*	0.175
December ^{Y} -February	-0.045	-0.019	-0.064
decimal latitude	0.196**	(1) 0.262**	0.108
decimal longitude	-0.007	-0.006	0.112

Significance: **P <0.01; * P <0.05 (one-tailed), **Symbols**: ^{Y} = prior year;
[1] [2] [3] = order in stepwise (forward) regression

Table 5-3. Correlations between temperature, precipitation, latitude, longitude and flight date for the museum and observation data for *Deilephila elpenor*. Correlations are shown with mean temperatures for 3-monthly periods and individual months in the same year as flight (January- August).

	D.elpenor	
	Museum (BM) (1893-1992) *n = 45*	Observation (Wicken Fen) (1899-2009) *n =102*
Period of temperature mean:		
July	0.079	0.117
June	-0.137	-0.153
May	-0.300*[1]	-0.041
April	-0.088	-0.012
March	-0.139	-0.209* [1]
February	0.119	-0.081
January	-0.079	0.022
3 month means:		
March - May	-0.287*	-0.149
December ^{Y}– February	0.078	-0.049
Period of precipitation mean:		
May	0.103	0.093
April	0.085	-0.134
March	-0.202	-0.004
February	-0.035	0.017
January	-0.197	0.179*
3 month means:		
March - May	-0.070	-0.044
decimal latitude	-0.013	-
decimal longitude	-0.290*	-

Significance: **P<0.01; *P<0.05 (one-tailed);
Symbols: ^{Y} = prior year: [1] = order in stepwise forward regression

5.2.3 Museum data

5.2.3.1 Euclidia glyphica

All 442 specimens of *Euclidia glyphica* collected in Britain and held at the Natural History Museum, London were examined. One hundred and sixty specimens had tags which were accessible and legible. Four specimens were collected in Ireland, and 5 originated in Wales: these were discarded. The remaining 151 were collected in England and for these specimens location, vice county, and collectors were recorded. Specimens with incomplete collection date were discarded. Forty five percent of the 151 tagged specimens were rejected; 30 were imprecisely dated (only the month or year), 3 specimens did not have a clear location, 34 specimens were duplicates collected on the same and at the same location by a single collector and one specimen was marked as bred. The final data set comprised 83 specimens providing at least one data point for each of 45 years, spanning an 86-year period from 1887 to 1972 (Table 5-1). Peaks of collection activity were evident during the 1890s and again during the 1940s (Fig. 5-3a).

5.2.3.2 Melanargia galathea

All 676 specimens of *Melanargia galathea* collected in Britain and held at the Natural History Museum, London were examined. Two hundred and seventy nine specimens had tags which were accessible and legible. All of these specimens were collected in England, mainly from southern and central vice counties. For these specimens location, vice county, and collectors were recorded. Specimens with incomplete collection date were discarded. Forty one percent of the 279 tagged specimens were rejected; 27 were imprecisely dated (only the month or year), 11 specimens did not have a clear location, and 80 specimens were duplicates collected on the same and at the same location by a single collector. The final data set comprised 161 specimens (94 male, 67 female) providing at least one data point for each of 61years, spanning a 109-year period from 1890 to 1998 (Table 5-2).

5.2.3.3 Deilephila elpenor

All specimens of *Deilephila elpenor* collected in England and held at the Natural History Museum, London were examined. 80 specimens had tags which were

accessible and legible. Location, vice county, and collector were recorded. Specimens with incomplete collection date were discarded. Forty four percent of the 80 tagged specimens were rejected: 20 were imprecisely dated (only the month or year), one specimen, though dated, did not have a clear location, 8 specimens were duplicates collected on the same and at the same location by a single collector and 6 specimens were marked as bred and were not used. The final data set comprised 45 specimens providing at least one data point for each of 30 years, spanning a 100-year period from 1893 to 1992 (Table 5-3),(Fig. 5-3b).

5.2.4 National Biodiversity Network (NBN) data for Euclidia glyphica.

Field observation data for *Euclidia glyphica* was accessed via the NBN website http://data.nbn.org.uk/interactive/map.jsp?srchSp=MM0001Z100DH5JN1

Six hundred and forty nine observation records were examined. Seventy eight percent of the records were discarded; 442 were not adequately dated, giving only the month, year or year range; 65 records were duplicates collected on the same date and at the same location by a single collector, and one record was dated in November, well beyond the accepted period of flight activity and almost certainly an error. The final data set comprised 141 specimens providing at least one data point for each of 35 years, spanning a 55 year period from 1956 to 2010 (Table 5-1), (Figure 5-3a).

5.2.5 Wicken Fen, National Nature Reserve Cambridgeshire

(Grid reference TL 562 705; Latitude 52.310818; Longitude 0.29132301)

A remaining area of the once extensive Cambridgeshire Fens, the site has been owned by the National Trust since 1899 (Fig. 5-1a,b). More than 7800 species have been recorded at the reserve, making it one of the most species-rich reserves in the UK. It has Site of Special Scientific Interest (SSSI) recognition under the Wildlife and Countryside Act 1981, has been accorded Wetland protected under the international Ramsar Convention and designated a Special Area of Conservation under the EU Habitats Directive http://www.wicken.org.uk/index.html.

Observation data for *Deilephila elpenor* recorded at Wicken Fen was accessed via the National Biodiversity Network (NBN) website http://data.nbn.org.uk/interactive/info.jsp?srchSp=24848.
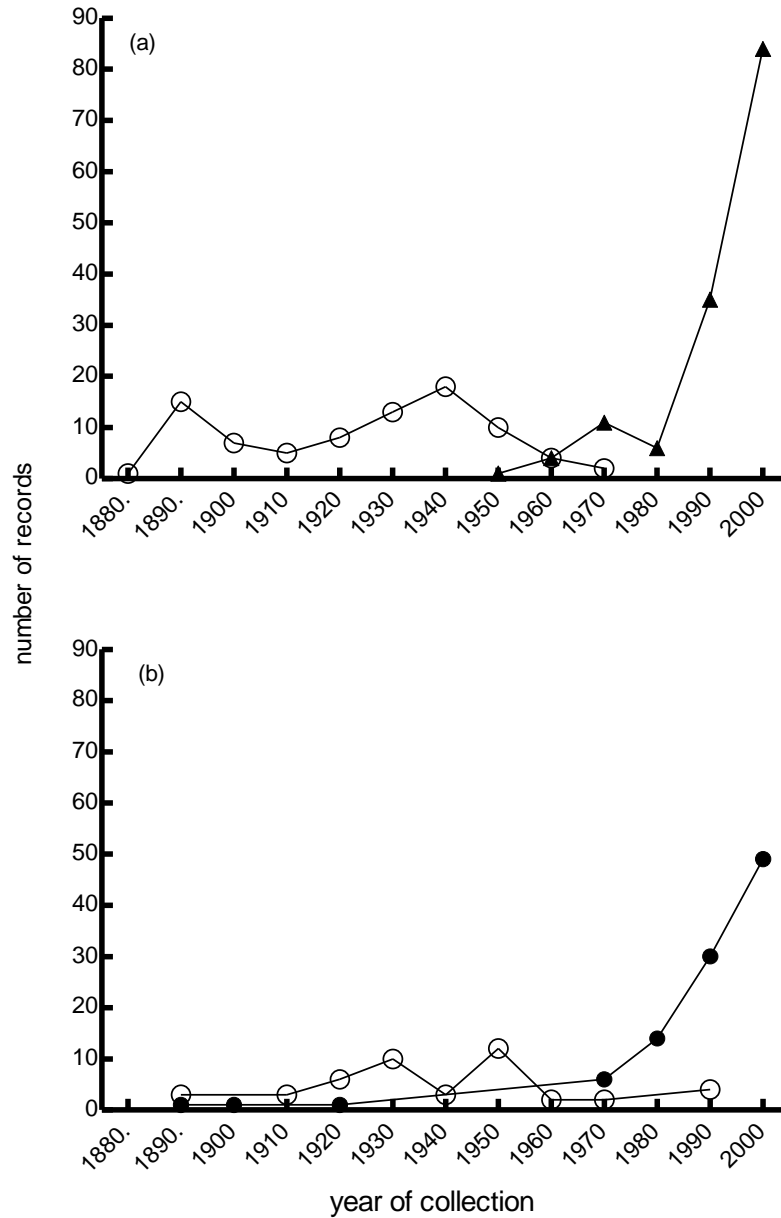
Figure 5-3. Collection over time of museum specimens and observation data since 1880 for (a) *Euclidia glyphica* and (b) *Deilephila elpenor*.
Symbols: o = museum data held at the Natural History Museum, London (BM), ▲ = observation from the National Biodiversity Network, ● = observation data from Wicken Fen, Cambridgeshire (National Trust)

All 118 observation records at the site were examined. Fourteen percent of the records were discarded; 9 were not adequately dated and a further 7 specimens were duplicates collected on the same date and at the same location by a single collector. From the collectors' notes most of the specimens appear to have been collected via overnight light trap. The final data set comprised 102 specimens providing at least one data point for each of 33 years, spanning a 111-year period from 1899 to 2009. The vast majority (97%) of the observations at Wicken Fen, Cambridgeshire were gathered between 1970 and 2009 (Fig. 5-3b).

5.2.6 Meteorological data

5.2.6.1 Central England Temperature

Mean monthly Central England Temperature (CET) records for the period 1820-2010 (Parker *et al*. 1992) were obtained from the UK Meteorological Office: http://www.metoffice.gov.uk/hadobs/hadcet/cetml1659on.dat. Three-month mean temperature data was calculated for each year (Winter-December (the prior year), January, February; Spring -March, April, May; Early-Summer -April, May, June; Mid-Summer -June, July, August).

5.2.6.2 England and Wales Precipitation

Mean monthly England and Wales's precipitation figures for the period 1820- 2010 were obtained from the Met Office website:

http://www.metoffice.gov.uk/research/hadleycentre/CR_data/Monthly/HadEWP_act.txt.

Mean seasonal precipitation figures were calculated for each year (Winter-December (the prior year), January, February; Spring-March, April, May; Mid-Summer-June, July, August).

5.2.6.3 Wicken Fen, Ely, Cambridgeshire - Local Climate Data

Local temperature and precipitation data, recorded at the National Institute of Agricultural Botany (NIAB), Huntington, Cambridgeshire (Latitude 52.221909, Longitude 0.096291222) between 1959 and 2011 was available from the Met Office website http://www.metoffice.gov.uk/climate/uk/stationdata/cambridgedata.txt. This

was the closest weather station to Wicken Fen at a distance of *c.*10 miles. Records from this weather station were used to investigate correlations of sighting data of *Deilephila elpenor* (Elephant Hawkmoth) at Wicken Fen between 1970 and 2009 with temperature and precipitation variables. Temperature records comprised monthly minimum and maximum temperatures. These were used to calculate mean monthly temperature.

5.2.7 Geographical effects

Chapter 3, Section *3.2.4* provides a description of the method of geo-referencing specimens.

5.2.8 Analysis of museum, herbarium and field data

The distribution of collection dates in the museum and herbarium datasets (*Euclidia glyphica*, *Deilephila elpenor*, *Anacamptis pyramidalis*, *Platanthera bifolia* and *P. chlorantha*) and the field observation datasets (*E. glyphica* and *D. elpenor*) were expressed as number of days after 1 March. Datasets were checked for normality and presence of outliers. All sets of phenology data were examined for correlations with mean CET temperature data from January to July or August in the flowering or flight season. Where appropriate these data included mean monthly temperature and its averages over successive 3-month periods (Winter-December–February; Spring-, March–May; Early-Summer-April-June; Mid-Summer-June-August).

All sets of phenology data were also examined for correlations with mean precipitation data from January to July in the flowering or flight season. These data included mean monthly precipitation and its averages over successive 3-month periods (Winter-December–February; Spring-, March–May).

Correlations and regression analyses were carried out with SPSS 18 (IBM Corp., 1 New Orchard Road, Armonk, NY, USA). Median, quartiles and range were calculated as described by Sokal & Rohlf (1969) ; slopes and intercepts of regressions were compared via analysis of covariance (ANCOVA) (Zar 1984) using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com.

5.3 Results

5.3.1 The Anacamptis pyramidalis-Euclidia glyphica *pollination mode*

Data derived from museum specimens over the period from 1887 until 1972, and recorded in the field between 1956 and 2010, both confirmed the importance of spring temperature in determining flight time of *Euclidia glyphica*. Significant correlations were found between flight date and several measures of mean temperature in the CET records in the preceding months (Table 5-1).

For museum material, there were significant negative correlations with mean temperature in April, May and June of the year of flight and with mean temperature over the 3-month periods March to May, and April to June (Table 5-1). The strongest correlation was with mean temperature for May. There were also significant positive correlation between mean precipitation for April and flight time (Table 5-1).

Results for the field data were similar; the strongest correlation with a single month was with mean temperature for May. Whilst there were significant negative correlations between flight date and mean temperature for January, February and March (Table 5-1), the highest correlation was with the mean for the 3-month period March-May. There was also a significant negative correlation with mean winter temperature (December-February) and a significant negative correlation with mean EWP for June. The geographic range of collection activity for museum records and for field observation was comparable (Table 5-4). Combining the museum and field data flagged significant correlations with mean monthly temperatures for April, May and June, whilst the strongest correlation was with the mean for the 3-month period April-June (early summer) (Table 5-1).

As expected, warmer springs were associated with earlier flight of *E. glyphica*. The regression of flight date obtained from the museum specimens on mean March-May (spring) temperature (Fig. 5-5a) accounted for 6% of the variation in flight time. A 1 °C increase in mean spring temperature was associated with an advance in flight of 5.7 days. Analysis of the field data yielded comparable results. Linear regression of flight date on mean spring temperature accounted for 10% of the variation in flight time (Fig. 5-5b) and a 1 °C increase in mean spring temperature was associated with an advance

in flight of 7.6 days. The regression models derived from the museum data and field data were statistically indistinguishable: neither the gradients ($F_{1,220} = 0.3483$, $P = 0.556$) nor the intercepts ($F_{1,221} = 3.2377$, $P = 0.073$) were significantly different, demonstrating that the phenological response to temperature was the same during the different periods over which the two sets of data were collected. Combining the museum and field datasets the regression of flight date on mean spring temperature accounted for 7% of the variation in flight time (Fig. 5-5c) and a 1 °C increase in mean spring temperature was associated with an advance in flight of 5.8 days.

Table 5-4. Range of geographic coordinates (latitude and longitude) of museum collections and field observation of *Euclidia glyphica*, *Melanargia galathea* and *Deilephila elpenor*. BM and NBN records are given as vice county centroids; Wicken Fen, Cambridgeshire is given as an exact location.

	Euclidia glyphica		*Melanargia galathea*	*Deilephila elpenor*	
	Museum BM	Observation NBN	Museum BM	Museum BM	Observation Wicken Fen
Decimal latitude:					
Maximum	53.3609	54.7254	54.0322	54.0322	52.3182
Minimum	50.6039	50.8044	51.0241	50.8522	52.3182
Range (decimal °)	2.7570	3.9210	3.0081	3.1800	-
Decimal longitude:					
Maximum	1.2979	1.3418	1.5279	1.5777	0.2923
Minimum	-3.6512	-3.0399	-4.0976	-5.0279	0.2923
Range (decimal °)	4.9491	4.3817	5.6255	6.6056	-

Analysis of the herbarium data for *Anacamptis pyramidalis* yielded similar results. Linear regression of flowering date on mean spring temperature accounted for 15% of the variation in flowering time (Fig. 5-5d) and a 1 °C increase in mean spring temperature was associated with an advance in flowering of 7.1 days. The slopes of the regression models derived from the combined museum and field data for *E. glyphica* and the herbarium data for *A. pyramidalis* were statistically indistinguishable ($F_{1,371} = 0.3655$, $P = 0.546$), although the differences between the intercepts were significant ($F_{1,372} = 301.062$, $P < 0.001$).
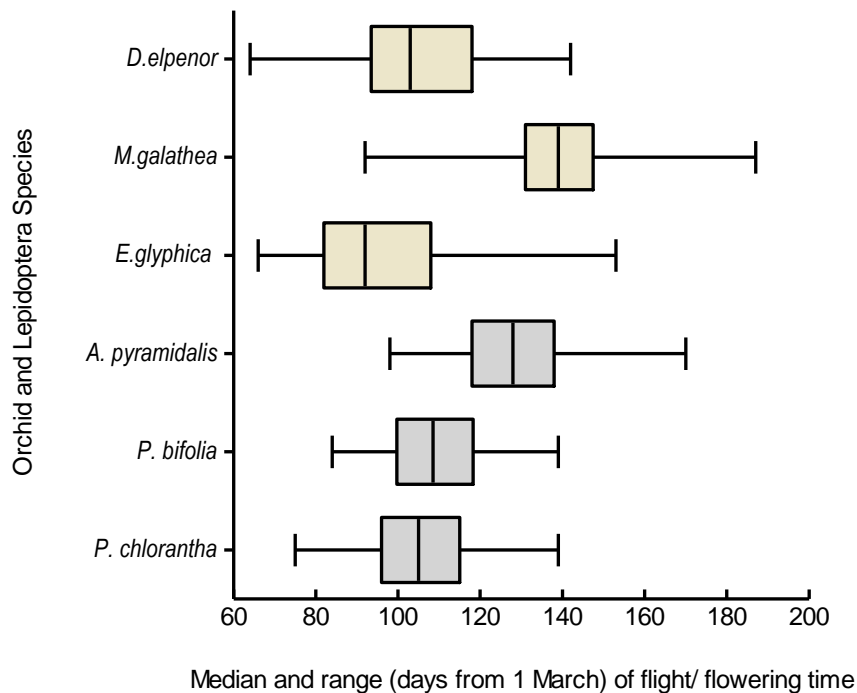
Figure 5-4. Median, 25 and 75 percentiles and range of; flight date for museum records of *D.elpenor*, *M. galathea* and *E.glyphica* and; flowering date for herbarium specimens of *A.pyramidalis*, *P.bifolia* and *P.chlorantha*.

Warm temperatures in early summer were also associated with earlier flight. The regression of flight date obtained from the museum specimens on mean April-June (early summer) temperature (Fig. 5-6a) accounted for 20% of the variation in flight time. A 1 °C increase in mean temperature in early summer was associated with an advance in flight of 11 days. For the field data linear regression of flight date on temperature for early summer accounted for 7.3% of the variation in flight time (Fig. 5-6b) and a 1 °C increase in mean temperature was associated with an advance in flight of 7.6 days. The slopes of the regression models derived from the museum data and field data were statistically alike ($F_{1,220} = 0.9955$, $P = 0.3195$), however the difference between the intercepts was significant ($F_{1,221} = 5.0214$, $P = 0.026$).
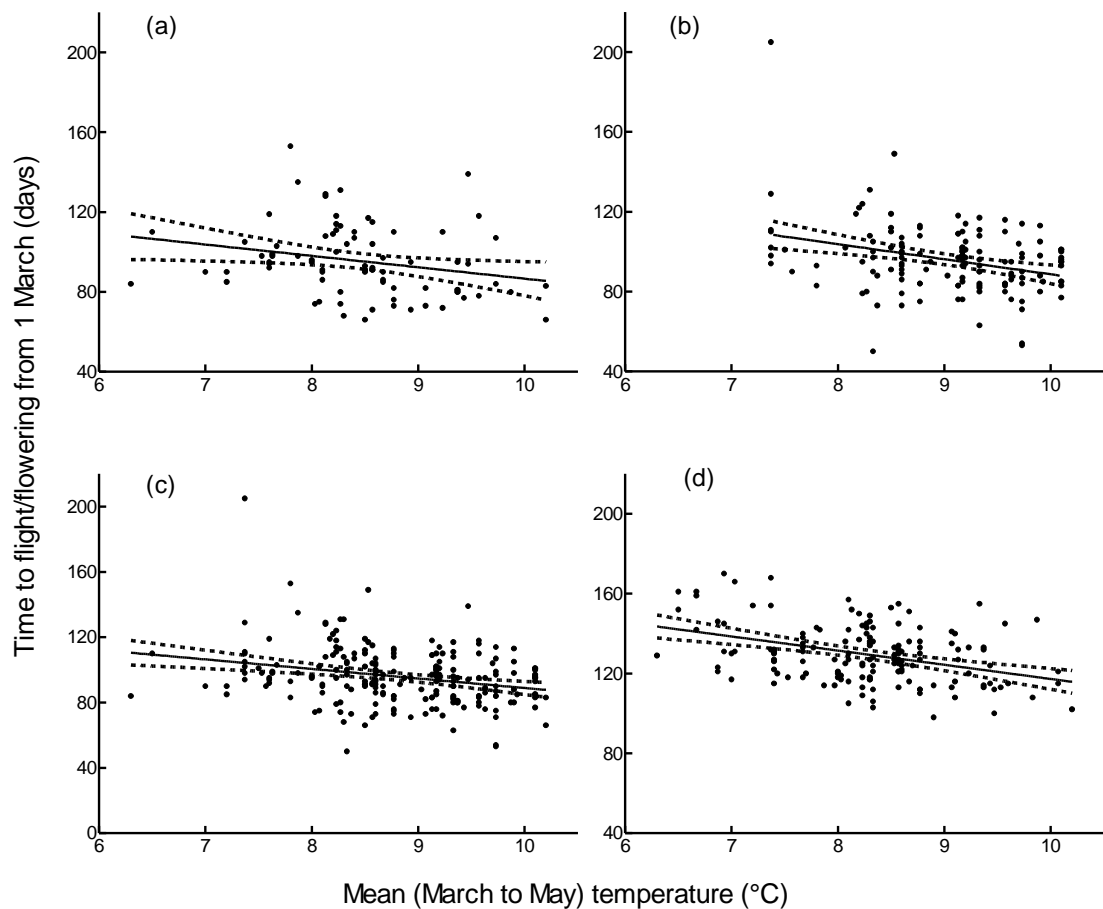
Figure 5-5. Relationship between flight date (days after 1 March) and mean (March-May) temperature for (a) museum specimens of *E. glyphica* ($y = 143.6$ [± 21.33] $- 5.7x$ [± 2.51], $r^2 = 0.06$, $P = 0.026$, $n = 83$) ; (b) field data for *E. glyphica* ($y = 164.2$ [± 17.74] $- 7.56$ [± 1.96], $r^2 = 0.097$, $P < 0.001$, $n = 141$); (c) combined museum and field data for *E. glyphica* ($y = 147.3$ [± 12.85] $- 5.84x$ [± 1.45], $r^2 = 0.07$, $P < 0.001$, $n = 224$); and (d) flowering date and mean (March-May) temperature for herbarium specimens of *A. pyramidalis* ($y = 188.3$ [± 11.52] $- 7.1x$ [± 1.39], $r^2 = 0.15$, $P < 0.001$, $n = 151$).

The regression of flight date obtained from the combined museum and field data for *E. glyphica* on early summer temperature (Fig. 5-6c) accounted for 10% of the variation in flight time. A 1 °C increase in mean temperature for early summer was associated with an advance in flight of 7.8 days. For herbarium data of *A. pyramidalis* the regression of flowering date on mean temperature for early summer accounted for 22%

of the variation in flowering time (Fig. 5-6d) and a 1 °C increase in mean early summer temperature was associated with an advance in flowering of 11.3 days. The slopes of the regression models derived from the combined museum and field data for *E. glyphica* and the herbarium data for *A. pyramidalis* were statistically alike: ($F_{1, 371} = 1.9747$, $P = 0.161$), however the intercepts ($F_{1, 372} = 297.402$, $P < 0.001$) were significantly different. Similarly the slopes of the regression models derived from the museum data for *E. glyphica* and herbarium data for *A. pyramidalis* were statistically identical ($F_{1,230} = 0.0068$, $P = 0.9342$), however the differences between the intercepts were significant ($F_{1,231} = 277.55$, $P < 0.001$).

5.3.2 The Anacamptis pyramidalis-Melanargia galathea *pollination mode*

Data derived from museum specimens over the period from 1890 until 1998 confirmed the importance of mid-summer (June-August) temperature in relation to flight time for *Melanargia galathea*. Warm temperatures during summer months June to August were associated with earlier flight. Data for males and females combined showed significant negative correlations between flight date and mean temperature for June, July and August in the CET records and with mean temperature over this 3-month period (Table 5-2) which reflects the flight period of *M. galathea* in the UK (Fig. 5-4) There was also a significant positive correlation between flight time and mean precipitation for March (Table 5-2).

The regression of flight date obtained from the museum specimens on mean June - August (mid-summer) temperature (Fig. 5-7a) accounted for 6% of the variation in flight time. A 1 °C increase in mean temperature for mid-summer was associated with an advance in flight of 4.3 days.

For herbarium data of *Anacamptis pyramidalis* the regression of flowering date on mean June- August temperature accounted for 17% of the variation in flowering time (Fig. 5 -7b) and a 1 °C increase in mean mid-summer temperature was associated with an advance in flowering of 8.5 days. The slopes of the regression models derived from the museum data for *M. galathea* and the herbarium data for *A. pyramidalis* were significantly different: ($F_{1, 308} = 4.3962$, $P = 0.0368$).
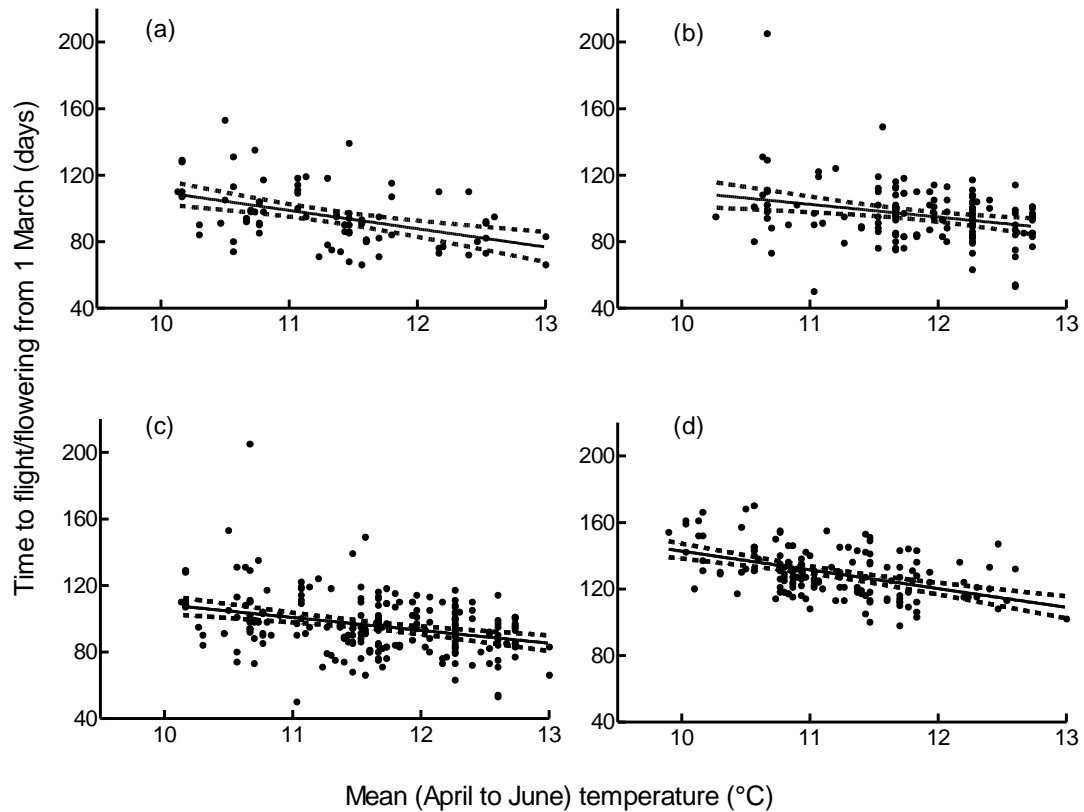
Figure 5-6. Relationship between flight date (days after 1 March) and mean (April-June) temperature for (a) museum specimens of *E. glyphica* (y = 220.2 [± 28.1] − 11.03x [± 2.48], r^2 = 0.20, P < 0.001, n = 83) ; (b) field data for *E. glyphica* (y = 186.3 [± 27.41] − 7.62 [± 2.31], r^2 = 0.073, P < 0.01, n = 141); (c) combined museum and field data for *E.glyphica* (y = 186.0 [± 18.58] − 7.75x [± 1.59], r^2 = 0.10, P < 0.001, n = 224); and (d) flowering date and mean (April - June) temperature for herbarium specimens of *A. pyramidalis* (y = 255.5 [± 19.6] − 11.27x [± 1.75], r^2 = 0.22, P < 0.001, n = 151).
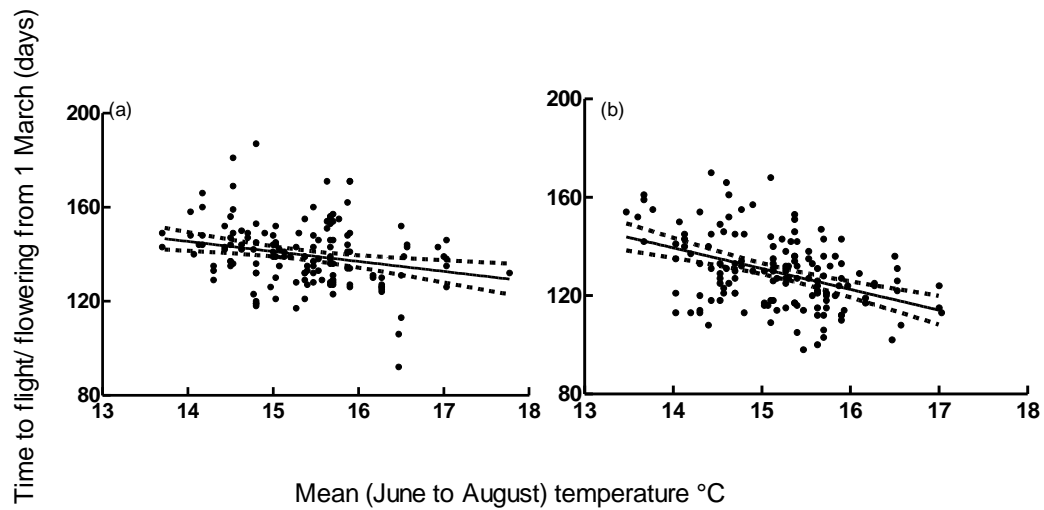
Figure 5-7. Relationship between (a) flight date (days after 1 March) and mean (June - August) temperature for museum specimens of *M. galathea* ($y = 205.0$ [$\pm$ 20.21] $- 4.25x$ [$\pm$ 1.32], $r^2 = 0.06$, $P < 0.01$, $n = 161$) and (b) flowering date and mean (June – August) temperature for herbarium specimens of *A. pyramidalis* ($y = 257.9$ [$\pm$ 23.14] $- 8.46x$ [$\pm$ 1.52], $r^2 = 0.17$, $P < 0.001$, $n = 151$).

5.3.3 The Platanthera spp.-Deilephila elpenor *pollination mode*

For museum material of *Deilephila elpenor*, there were significant negative correlations between flight date and mean CET for May, mean CET over the 3-month period March-May, and decimal longitude of collection site. No significant correlation was found between any measure of precipitation and flight time (Table 5-3). For field data gathered at Wicken Fen, Cambridgeshire there was a significant negative correlation with mean CET in March and a significant positive correlation with mean precipitation for January (Table 5-3). No significant correlations were found between flight time and any temperature or precipitation variable derived from the weather station at NIAB, Cambridgeshire. The field and museum datasets for *D. elpenor* were dissimilar in both geography and time. Whilst 97% of the data at Wicken Fen, Cambridgeshire, was gathered between 1970 and 2009, more than 91% of the museum data was collected prior to 1970 (Fig. 5-3b). Museum data was collected over a range of 3.18 decimal latitude and 6.61 decimal longitude (Table 5-4), compared to the single site data of Wicken Fen.
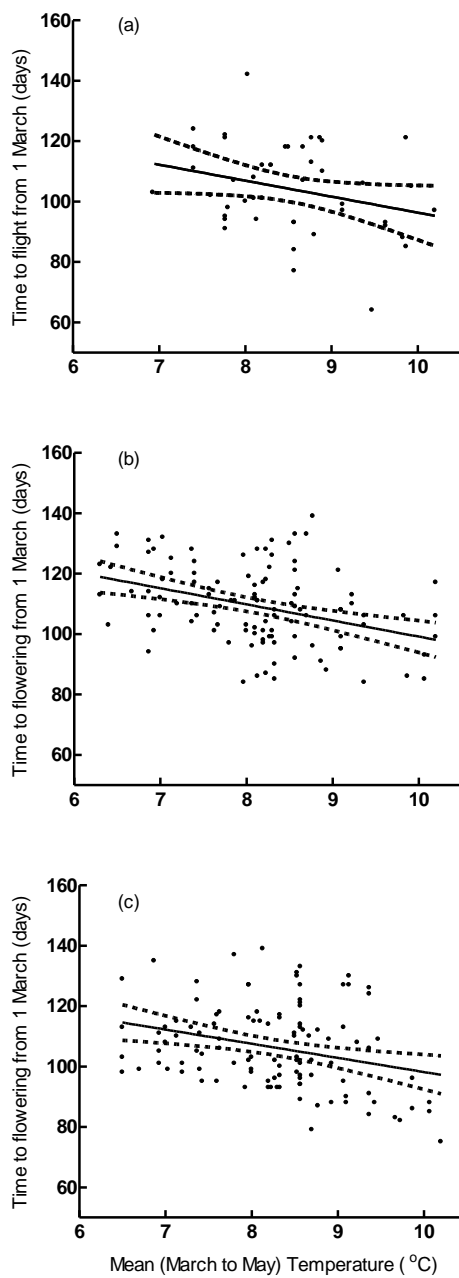
Figure 5-8. Relationship between flight date (days after 1 March) and mean spring (March – May) temperature for (a) museum specimens of *Deilephila elpenor* ($y = 149.3$ [$\pm$ 23.03] - $5.29x$ [$\pm$ 2.69], $r^2 = 0.083$, $P = 0.055$, $n = 45$); (b) flowering date and mean spring temperature for *Platanthera bifolia* ($y = 152.6$ [$\pm$ 10.44] - $5.349x$ [$\pm$ 1.28], $r^2 = 0.14$, $P < 0.001$, $n = 110$) and (c) flowering date and mean spring temperature for *Platanthera chlorantha* ($y = 145.1$ [$\pm$ 12.51] - $4.687x$ [$\pm$ 1.5], $r^2 = 0.083$, $P < 0.001$, $n = 109$).

Correlation between flight date and CET was earlier in the season (March) at the Wicken Fen site, than for the museum data (May) (Table 5-3). Wicken Fen is toward the east of the species' range and, given the significant negative correlation with easting, this would be expected.

Warmer springs were associated with earlier flight of *D. elpenor*. Using museum data, the regression of flight date on mean spring (March-May) temperature (Fig. 5-8a) accounted for 8.3% of the variation in flight time, and a 1 °C increase in the temperature mean was associated with an advance in flight of 5.3 days. These results, based on a small set of 45 specimens, bordered significant ($P = 0.0555$). Analysis of the herbarium data for *Platanthera bifolia* yielded comparable results. Linear regression of flowering date on mean spring temperature accounted for 14% of the variation in flowering time (Fig. 5-8b) and a 1 °C increase in the temperature mean was associated with an advance in flowering of 5.3 days. The regression models derived from the museum data for *D. elpenor* and the herbarium data for *P. bifolia* were statistically indistinguishable, neither the gradients ($F_{1,151} = 0.0004$, $P = 0.984$) nor the intercepts ($F_{1,152} = 1.578$, $P = 0.211$) were significantly different. As expected, analysis of the herbarium data for *P. chorantha* data yielded very similar results. Linear regression of flowering date on mean spring temperature accounted for 8.3% of the variation in flowering time (Fig. 5-8c) and a 1 °C increase in the temperature mean was associated with an advance in flowering of 4.7 days. The regression models derived from the museum data for *D. elpenor* and the herbarium data for *P. chlorantha* were statistically identical, neither the gradients ($F_{1,150} = 0.0403$, $P = 0.841$) nor the intercepts ($F_{1,151} = 0.1409$, $P = 0.708$) were significantly different.

5.4 Discussion

Few prior studies have examined the phenological relationship between plants and their pollinators (Doi, Gordo & Katano 2008) and this is the first study to quantify and compare temporal variation in the flowering date of orchid species and flight date of some of their Lepidopteran pollinators. To date studies of Lepidoptera phenology have been limited, by lack of long-term data (Woiwod 1997; Roy & Sparks 2000). Museum collections provide a valuable additional source of such data and this research is the

first study to provide a validation that justifies the use of these extensive historical collections.

As anticipated there was substantial wastage in the museum data due to duplication of collection, the incidence of incomplete data recording and an unwillingness to allow specimens to be moved when labels were obscured. The loss of data did not detract from their overall value; sufficient records were available to produce normally distributed data and to flag significant correlations with various measures of mean temperature. In the main, mean monthly precipitation generated small positive correlations with flight date, concurring with prior findings (Stefanescu, Penuelas & Filella 2003).

Using museum and field data this study quantified the flight phenologies of three native, univoltine Lepidoptera in relation to monthly CET temperature records, and compared results to the flowering phenologies of three orchid species, each of which relies on Lepidopteran pollinators. The species selected were either known pollinator species or a suitable proxy for potential pollinators.

The robustness of the museum record for phenological research as a measure of flight time was demonstrated using the species, *Euclidia glyphica*, as relationships derived from museum and field data were statistically identical. Comparison of museum and observation data for *E. glyphica* gave strikingly similar results notwithstanding that the datasets differed in length and were separated in time. *E. glyphica* is largely confined to southern England and the geographic ranges of the museum and field datasets were analogous. The slopes of the regression models on mean spring (March-May) and mean early summer (April-June) temperature were both statistically indistinguishable between museum and field data. This enabled the two sets of data to be justifiably combined into a single dataset. The value of herbarium specimens to examine flowering phenology had been previously validated, using the orchid *Ophrys sphegodes* (Robbirt *et al.* 2011).

Darwin (1877) named *E. glyphica* as a pollinator of *Anacamptis pyramidalis*, and the species is often seen in association with the Burnet moths, which are also known pollinators of *A. pyramidalis*. *E. glyphica* flies only during the earlier period of flowering of *A. pyramidalis* but response to variation in temperature of spring and

early summer was statistically indistinguishable for plant and pollinator. A 1 °C rise in mean temperature during early summer was associated with an advance in flight of 7.8 days and an advance in flowering of 11.3 days.

These results demonstrate that averaged three-monthly temperature means can provide an apt temperature variable for phenological study, echoing prior studies (Pollard 1988; Roy & Sparks 2000; Gordo & Sanz 2006) Whilst much of the variance in flight phenologies could not be explained, the regressions of flight data on mean spring temperature produced results consistent with prior studies. Gordo & Sanz (2006) found that mean temperature between February and April was a significant predictor of appearance and that higher spring temperatures were significantly associated with earlier flight. Data gathered over a 23 year period (1976-1998), based on BMS field observation of 35 butterfly species demonstrated advances in first and peak flight dates associated with spring and summer temperatures, and an advance of 2-10 days per 1°C rise in temperature (Roy & Sparks 2000). Similarly Sparks & Yates (1997) demonstrated a significant relationship between spring temperature and first appearance dates in 12 common British butterflies, and estimated an advance in first flight of 3 to 10 days per 1°C rise in temperature.

In a study of two resident British butterfly species, *Maniola jurtina* and *Pyronia tithonus,* Brakefield (1987) found that higher temperatures during June to August were associated with earlier flight dates. Both species, members of the family Satyridae, are single generational in the UK (Asher *et al.* 2001), and flight periods are similar to *Melanargia galathea*, being June-September for *M. jurtina* (Novák 1980) and July-August for *P. tithonus* (Asher *et al.* 2001).

M. galathea and *A. pyramidalis* share a common geographic range and habitat preference in the UK. Whilst *M. galathea* is not named in the literature as a pollinator of *A. pyramidalis* it has been recorded visiting the orchid (Fig. 5-2a).It is probable that *M. galathea* is one of a suite of pollinators of *A.pyramidalis* in the UK, and it represents a suitable proxy for potential pollinators. Mean date of flight of *M. galathea* is marginally earlier than mean date of flowering of *A.pyramidalis* but flowering range falls wholly within the butterflies' flight range.

Using data from the Rothamsted Insect Survey (1976-1995) for 18 univoltine moth species across 6 sites in the UK, Woiwod (1997) demonstrated a significant tendency for negative regressions in flight phenology with time over the twenty year period. A significant positive regression was evident for a later emerging species, *Xestia xanthographa*, suggesting that late season species might be expected to emerge later with higher temperatures (Woiwod 1997).

The potential for asynchrony between plant and pollinator was apparent between *A. pyramidalis* and the mid-season butterfly *M. galathea*. For *M. galathea* flight advanced with increases in mean temperature for the 3-month period June to August (summer), but this advance was significantly less than the advance in flowering of *A. pyramidalis*. Based on these regressions the phenologies of plant and pollinator would be expected to become increasing asynchronous at elevated temperatures. *A. pyramidalis* is a non-rewarding pollinator generalist and asynchrony between flowering and flight phenologies of potential pollinators might be expected with climate warming because species respond differentially to climate cues and in a non-rewarding pollination system convergent selective forces are absent. Conversely, there was no statistical evidence of asynchrony between the phenologies of *A. pyramidalis* and *E. glyphica*, which flies during the earlier period of its flowering.

Surprisingly local temperature data for Wicken Fen, Cambridgeshire did not generate any significant correlations with local observation data of *Deilophila elpenor*. This unexpected result is probably explained by the lack of resolution founded in the use of minimum and maximum temperatures averaged on a monthly rather than daily basis and which may have been too imprecise to pick up any phenological signal.

Using CET meteorological data for analysis, this research found that the phenology of flowering of the rewarding orchids *Platanthera bifolia* and *P. chlorantha* were closely synchronised with the flight phenology of the key pollinator *D. elpenor,* suggesting that these species are likely to maintain phenological synchrony with further modest rises in mean spring temperature. Since *P. bifolia* and *P. chlorantha* are rewarding orchids and pollinator specialists, convergent selective pressures would be expected to support phenological synchrony. Further work is necessary to establish whether

rewarding or non-rewarding pollination systems orchids are differentially susceptible to potential plant-pollinator asynchrony.

For pollinator generalists such as *A. pyramidalis*, the impact of disruption to current phenological patterns may be mitigated by the potential for new pollinator relationships to emerge due to demographic shifts. Sparks, Roy & Dennis (2005) found that elevated temperatures in Europe substantially increased migration of Lepidoptera to Britain. Pollard (1991) found that range expansion was associated with advance in mean flight date and extension of the flight period for *Pyronia tithonus* in the UK but did not investigate causal factors. Habitat fragmentation may however constrain range expansion for some species (Hill, Thomas & Huntley 1999) and may lead to a decline in abundance of habitat specialists (Warren *et al.* 2001) relative to habitat generalists.

Given that phenological and demographic changes of Lepidoptera in response to climate warming are complex and difficult to predict any evaluation of the impact of climate warming on plant-pollinator relationships needs to be species-specific and mindful of confounding factors. In this research the study species are all univoltine, facilitating identification of phenological trends. For multi-brooded species the wider range in flight period dampens the signal to noise ratio, making trends more difficult to identify and may further be confounded by an extension of flight period into the autumn. Consideration should also be made of the uncertainty in the degree of fidelity in many orchid-Lepidoptera pollinator associations. For some orchid species the suite of available pollinators is likely to be influenced by changes in range limits, habitat, and population abundance as well as phenology.

In conclusion, data derived from herbarium, museum specimens and field observation validates museum specimens as a source of phenological data for Lepidoptera, establishes phenological responses to temperature for Lepidoptera, and allows comparison with that of orchids. Warmer spring and summer temperatures were associated with advances in mean flowering and in mean flight activity. These results suggest that under current rates of climate warming in the UK flowering phenologies of the early-summer flowering orchids in this study and flight phenologies of the associated Lepidoptera pollinators studied should remain generally in synchrony. In contrast, for the mid-summer flowering orchid *Anacamptis pyramidalis* asynchrony

between flowering and flight activity of a potential pollinator *Melanargia galathea* is probable with further climate warming.

These results reaffirm the need for detailed knowledge at species level, toward understanding the consequences of climate-driven phenological shifts for plants and their pollinators.

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Chapter 6

Concluding remarks

6.1 The role of herbarium and museum collections in phenological study

A central aim of this thesis was the evaluation of historical herbarium and museum collections as a source of long-term phenological data for climate change research. Phenological studies, often with low signal-to-noise ratios, require sufficiently long-term data to elucidate any underlying trend. The choice of species for long-term study of phenology has, however, been dictated by the availability of suitable records. These are often scarce due to the overall shortage of long-term monitoring schemes (Sparks & Carey 1995). Often field monitoring is based on observation of first flowering dates, but these may not be optimum measures of plant responses to climate change as the data represent an extreme of the flowering distribution and are more susceptible to confounding effects than peak flowering (Miller-Rushing, Inouye & Primack 2008). Prior studies have suggested that herbarium data may provide a suitable proxy for field observation of flowering (Primack *et al.* 2004; Lavoie & Lachance 2006; Miller-Rushing *et al.* 2006) but this had not previously been validated. Field monitoring of peak flowering of the rare terrestrial orchid *Ophrys sphegodes* at a site in the UK provided an opportunity to corroborate the use of herbarium specimens as a proxy for field-based observational data of peak flowering time. The two datasets, showing statistically identical phenological responses to spring temperature, give credence to the use of herbarium data for phenological research.

For Lepidoptera, the Butterfly Monitoring Scheme has been generating valuable field-based phenological data of first, peak and last flight dates in the UK since the mid 1970s, although this is still a relatively short period with which to detect change (Roy & Sparks 2000). Museum specimens may represent valuable long-term records of insect flight phenology, especially where few field-based data exist, yet no prior studies have used museum data for this purpose. Extensive entomological collections held in museums in the UK, together with the more recent observational data gathered by members of the Bees Wasps and Ant Recording Society (BWARS), afforded the opportunity to verify museum records as a suitable proxy for long-term field data.

In validating the museum data, this research has established that the relationships between flight phenology and spring temperature for the Hymenoptera solitary bee *Andrena nigroaenea* were statistically identical when comparing museum records and field observations. This was also the case for the digger wasp *Argogorytes mystaceus*. There is little phenological data available with which to compare flight phenologies of Hymenoptera, although published data of temporal trends for the honey bee (Gordo & Sanz 2006) and wild bees (Bartomeus *et al.* 2011) suggest advanced flight with increased spring temperatures.

Similarly, for the moth *Euclidia glyphica* there was a statistically indistinguishable phenological response of peak flight date to mean spring temperature when comparing the museum records to the field-based records. For two further species of Lepidoptera, *Melanargia galathea* and *Deilephila elpenor*, the museum record showed that responses of peak flight time to spring temperature were within the ranges suggested by UK field-based studies (Sparks & Yates 1997; Roy & Sparks 2000).

For many species of plants and animals, biological collections are the only source of long-term phenological data. Two and a half billion specimens of flora and fauna are estimated to be held in these collections worldwide (Graham *et al.* 2004), representing an invaluable resource (Prather *et al.* 2004) for phenological studies which seek to understand and predict the consequences of continuing climate change.

6.2 Assessing flowering phenologies of British orchids

Using field observation, results of prior UK studies demonstrate that for many temperate plants, flowering advances with temperature (Fitter *et al.* 1995; Sparks, Jeffree & Jeffree 2000). Using data derived from herbarium specimens this thesis has clearly established the relationships between mean spring and summer temperatures and flowering time for the 15 orchid species in this study, representing more than a quarter of British species.

Flowering dates in spring and early-summer flowering orchid species were most strongly correlated with temperatures over the three to four months immediately prior to flowering. For each species, with the exception of *Gymnadenia conopsea* for which the data showed poor correlations, there were significant correlations between

flowering date and mean spring (March-May) temperature. For those species which flowered later in the season, seasonal temperature over spring and summer were equally important.

A recent study using 5,424 herbarium specimens for 41 orchid taxa collected in herbaria across Hungary between 1837 and 2009, found that, on average, flowering advanced over time, although advancement was significant for only nine species. Responses varied considerably between species, but trends were associated with pollination mode, life span, mean flowering time and geographical distribution. In general the strongest phenological responses of flowering over time were seen in the autogamous or deceptive orchids, and those flowering earliest in the season. Weakest responses were associated with nectar-rewarding and later flowering taxa (Molnár *et al.* 2012b).

In contrast to Molnár *et al.*, the aim of the research presented here was to explore the relationships between flowering time and various measure of temperature over the period of collection. Whilst this research was based on a smaller number of species there was no evidence that flowering response to temperature was influenced by reward or deceit as mode of pollination. In multiple regression analysis mean monthly temperature in March, April or May was flagged as a key variable for each orchid species irrespective of flowering time or pollination method. In linear regression of flowering response to mean spring temperature there were no significant differences in flowering phenology for 12 (92%) of the 13 orchid species for which this test was possible. For these 12 orchid species flowering advanced between 4.2 and 8.6 days for each 1°C increase in mean spring temperature.

The results presented in this thesis have established phenological signals of flowering response to temperature for nearly one third of orchid species in Britain, confirming a central hypothesis of this thesis that phenological responses of flowering time could be identified.

6.3 Assessing flight phenologies of pollinator species

Since insects are poikilothermic, development and flight activity are expected to be highly sensitive to temperature (Woiwod 1997; Gordo & Sanz 2006). Prior studies,

have established the importance of preceding temperature in the timing of insect flight activity, (Sparks & Yates 1997; Roy & Sparks 2000; Forister & Shapiro 2003; Stefanescu, Penuelas & Filella 2003; Dingemanse & Kalkman 2008; O'Neill *et al.* 2012).

The results presented in this thesis affirm the hypothesis that phenological signals of flight time could be identified from the museum record and concur with prior field studies: warmer spring and summer temperatures were associated with advances in peak flight activity. Peak flight of *Euclidia glyphica* advanced by *c.* 6 days, and *Deilephila elpenor* advanced by *c.* 5 days per 1°C rise in mean spring (March-May) temperature. *Melanargia galathea* advanced by *c.* 9 days per 1°C rise in mean summer (June-August) temperature. These findings fall within the range of results derived from the Butterfly Monitoring Scheme which suggest that flight phenologies would be expected to advance by between 2 and 10 days per 1°C rise in mean Central England Temperature (Roy & Sparks 2000).

For the two Hymenoptera species, *Andrena nigroaenea* and *Argogorytes mystaceus*, there are few phenological data available with which to compare flight phenologies, although published data of temporal trends for the honey bee (Gordo & Sanz 2006) and wild bees (Bartomeus *et al.* 2011) suggest advanced flight with increased spring temperatures.

6.4 Temporal plant-pollinator relationships: the potential for phenological asynchrony

The ultimate question of this thesis was whether there is evidence of potential asynchrony between the flowering phenologies of orchids in Britain and the flight phenologies of their pollinators. Phenological mismatch between species may occur if species respond differentially to climate change (Walther *et al.* 2002; Parmesan 2007). Relationships between species at different trophic levels are most vulnerable (Harrington, Woiwod & Sparks 1999; Thackeray *et al.* 2010) and in the UK there is evidence of systematic trophic-level asynchrony (Thackeray *et al.* 2010). There are, however, few prior phenological studies of plant-pollinator interactions (Hegland *et al.* 2009) and results are variable (Kudo *et al.* 2004; Gordo & Sanz 2005; Bartomeus *et al.* 2011).

Results presented in this thesis have demonstrated that in the highly specialised pollination system of the sexually deceptive orchid *Ophrys sphegodes*, the rate of advancement of peak flowering date and of overall peak flight activity of the pollinating bee *Andrena nigroaenea*, to mean spring temperature were statistically indistinguishable. However it is only the male bees that pollinate the orchid, whilst the females represent stronger competition than the flower for the copulatory attentions of the males. There are few data for this bee species but it is thought that the females mate only once and that male bees, emerging earlier than the females, have a lifespan of approximately 10 days, during which time they seek to maximize their mating opportunities (R. Paxton, pers. comm.). The results presented here show that males indeed emerge earlier than females and respond to temperatures earlier in the spring. Results also demonstrate an advance in peak flight of both males and females relative to peak flowering of *O. sphegodes*, with females also advancing relative to males. The earlier flight of the females suggests that peak flowering of the orchid may not only become increasingly out of synchrony with peak flight of the pollinating male, but may also become increasingly in competition with the female bee.

For the two closely related early summer-flowering rewarding orchids (*Platanthera bifolia* and *P. chlorantha*) peak flowering dates are likely to remain in synchrony with peak flight of a primary pollinator, the hawkmoth *Deilephila elpenor*. For the generalist, non nectar-rewarding orchid *Anacamptis pyramidalis,* synchrony with an early summer pollinator, *Euclidia glyphica*, is likely to be maintained with current rates of temperature rise. In contrast, synchrony with *Melanargia galathea*, a proxy for later flying pollinators, may become disrupted at elevated summer temperatures, should flowering continue to advance relative to peak flight.

Attempting to unravel the potential effects of climate change for plant-pollinator relationships is not easy and there is a myriad of confounding factors to consider. Climate change may also cause habitat fragmentation, create shifts in geographic range and altitudinal limits (Hill, Thomas & Huntley 1999): these will also influence outcomes for plants and pollinators. The vulnerability of plant-pollinator relationships to potential asynchrony also depends on the historical starting position. It may be erroneous to assume that species are presently in optimum synchrony, and hence

species that are not currently well adapted to their habitat may be vulnerable to small changes in synchrony (Parmesan 2007).

6.5 Suggestions for future work

Understanding the effects of climate change requires the accumulation of much detailed analysis of specific species and their ecological relationships (Stenseth & Mysterud 2002), and there is scope for further research amongst British orchids. It would be edifying to include additional species that rely on specific pollinators or pollinator groups. *Epipactis helleborine* is nectar-rewarding and is pollinated by wasps of the genus *Dolichovespul*a: self pollination is rare in the absence of pollinators (Foley & Clarke 2005). The extremely rare orchid *Ophrys fuciflora*, restricted in the UK to a few downland sites in south-east Kent, is thought to be pollinated by bees of the genus *Eucera.* Self pollination is rare whilst vegetative reproduction is not reported for this vulnerable species (Foley & Clarke 2005). Amongst the non nectar-rewarding orchids included in this thesis but not yet examined in relation to pollinators, *Orchis mascula* is pollinated primarily by naive newly emerged bumble-bee queens and hence timing of peak flowering of this very early flowering orchid may be an important factor for pollination success. For the nectar-rewarding, late-season flowering species *Spiranthes spiralis* and *Epipactis purpurata*, important pollinators are respectively bumblebees and vespid wasps; *Bombus pascuorum* and *B. lapidarius* (Willems & Lahtinen 1997); *Vespula austriaca* and *V. vulgaris* respectively (Jakubska-Busse & Kadej 2011) are named species. For these orchids synchrony of flowering and flight phenologies at the end of summer and in early autumn may be essential for pollination.

Results could be verified in terms of fruit set in the field. A strong phenological overlap between plant and pollinator would be expected to give higher fruit set relative to relationships showing a weak phenological overlap, and this could be investigated across multiple years. In turn, variation in fruit set patterns could be incorporated in population dynamics modelling.

An estimated 60 million records are already available for a wide range of taxa via internet information networks such as the Global Biodiversity Information Facility and HerpNET (Graham *et al.* 2004). On-line herbarium resources, such as the UK initiative

Herbaria United http://herbariaunited.org/ are becoming more widely offered. For all on-line resources it is important that images are also available so that users are able to verify taxonomy and assess flowering state.

One of the most extensive orchid collections to be available on-line is that of the herbarium of the University of Basel, Switzerland. Over 25,000 orchid specimens have been digitised from collections of Dr Jany Renz, and the Institute of Botany (University of Basel). This on-line herbarium is ideally placed to facilitate research into the relationships between flowering phenology, climate and altitude for European orchids. Historical temperature and precipitation data from meteorological stations across Switzerland, and at altitude, are also accessible from Meteo Schweiz http://www.meteoschweiz.admin.ch/web/en/services/data_portal.html.

It would be valuable to extend the study to orchid taxa of geographic regions beyond Europe. Little prior research has been undertaken in the Southern Hemisphere, although accessible long-term herbarium records and corresponding meteorological data exist. Using herbarium data previously received from the Herbarium of Western Australia, Perth, it would be instructive to assemble phenological datasets for selected orchids of the genus *Caladenia*. This genus is endemic to Western Australia, and typified by high pollinator specificity. Species within the genus are well researched: non-rewarding (sexual deception and non nectar-reward) are usual pollination modes.

It is probable that many plants change their pollinators over a sufficiently wide geographic area, and the interaction of plants and pollinators is therefore likely to alter across the landscape. Lepidopera are responding to climate change, and degraded habitat, not only in terms of phenological shifts but also in altered range limits (latitude and elevation) and population densities (Hill, Thomas & Huntley 1999; Parmesan *et al.* 1999; Warren *et al.* 2001; Franco *et al.* 2006; Thomas, Franco & Hill 2006). With sufficient data the aim of further research could include coupling phenological data with that of spatial shifts in order to develop a multi-dimensional model of asynchrony, identify potentially vulnerable plant-pollinator relationships across the landscape. Whilst pollination is of enormous ecological and economic significance and a responsive, and important phenological indicator of climate change

(Miller-Rushing, Inouye & Primack 2008), other species interactions could also be examined using natural history data, such as those of insect and food plant.

6.6 Conclusions

The results of this thesis represent the first validations of herbarium and museum data against field observation and affirm the value of the huge resources held in natural history collections for phenological research. The conclusions drawn enable the flowering phenologies of selected British orchid species to be placed in the context of peak flight activity of important pollinators. Prior studies suggest that plant-pollinator relationships might potentially become asynchronous if species respond differentially to climate warming. Species which are likely to be most vulnerable to the effect of asynchrony are those which are dependent on a single, or few, primary pollinators, and non-rewarding species whose pollinators are not subject to convergent selective pressure. Pollination of the sexually deceptive orchid *Ophrys sphegodes,* reliant on a single pollinator species, is a highly specialised model and findings of this thesis suggest a vulnerability to pollination disruption with further climate warming.

Additional research is needed to identify further specific plant-pollinator relationships that may be under threat, and there is scope to use the vast, presently under-utilised resources of natural history collections. Access to this data is improving at a pace with on-going digitisation of herbarium specimens and the expansion of on-line resources. These and similar databases are increasingly available worldwide and represent freely available, high quality data sources for phenological research. The validation presented in this thesis should help to unlock the vast resources of natural history collections, facilitating ecologists and climatologist toward greater knowledge of the ecological consequences of climate change.

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Appendix 1

Vice County Centroids for England and Wales: X,Y Values of Origin; Decimal
Latitude and Decimal Longitude.

V.C. No.	V.C. Name	X	Y	Decimal Latitude	Decimal Longitude
1	West Cornwall	167489.6605	39551.0045	50.2102	-5.2601
2	East Cornwall	215160.0587	70388.1332	50.5040	-4.6080
3	South Devon	283245.6551	79540.8969	50.6039	-3.6512
4	North Devon	258684.7564	115785.1530	50.9242	-4.0120
5	South Somerset	320430.0604	128336.8400	51.0488	-3.1365
6	North Somerset	354692.0849	149761.9029	51.2452	-2.6505
7	North Wiltshire	404478.4444	177672.9772	51.4980	-1.9369
8	South Wiltshire	405494.6235	141195.7923	51.1700	-1.9228
9	Dorset	376339.7402	100596.4461	50.8044	-2.3371
10	Isle of Wight	449818.7995	86470.7002	50.6758	-1.2963
11	South Hampshire	441379.0892	111950.2204	50.9055	-1.4129
12	North Hampshire	457910.1367	145435.8379	51.2052	-1.1725
13	West Sussex	502400.9381	116523.9945	50.9391	-0.5440
14	East Sussex	556037.2189	119409.0073	50.9530	0.2201
15	East Kent	603493.7662	149569.5229	51.2091	0.9119
16	West Kent	559449.4016	159041.0888	51.3082	0.2862
17	Surrey	513559.5111	153516.8462	51.2695	-0.3736
18	South Essex	572025.7862	193240.6075	51.6117	0.4831
19	North Essex	577567.6099	223911.1108	51.8855	0.5787
20	Hertfordshire	522849.1051	216030.5458	51.8294	-0.2187
21	Middlesex	519841.0816	184735.7954	51.5488	-0.2730
22	Berkshire	455170.9420	179746.9272	51.5140	-1.2064
23	Oxfordshire	449253.0766	212408.8179	51.8081	-1.2870
24	Buckinghamshire	483474.5594	214986.3287	51.8273	-0.7901
25	East Suffolk	628328.1478	264008.2390	52.2269	1.3418
26	West Suffolk	585841.9357	261697.2336	52.2221	0.7193
27	East Norfolk	623138.0145	311869.2226	52.6586	1.2979
28	West Norfolk	578813.9202	313700.9092	52.6915	0.6444
29	Cambridgeshire	545373.8553	273318.1743	52.3387	0.1324
30	Bedfordshire	506434.6992	243467.8557	52.0793	-0.4483
31	Huntingdonshire	521109.2251	277428.8164	52.3815	-0.2221
32	Northamptonshire	480243.0913	271128.7898	52.3324	-0.8238
33	East Gloucestershire	403812.7118	220183.8163	51.8802	-1.9460
34	West Gloucestershire	370372.1577	198179.2554	51.6816	-2.4299
35	Monmouthshire	334728.3821	201898.3149	51.7120	-2.9461
36	Herefordshire	348916.6855	245356.7796	52.1042	-2.7472
37	Worcestershire	388928.0174	260107.1054	52.2390	-2.1636
38	Warwickshire	428308.3668	270627.2601	52.3330	-1.5860
39	Staffordshire	397156.9996	325103.5680	52.8235	-2.0436
40	Shropshire	351729.0771	305145.7466	52.6419	-2.7148
41	Glamorganshire	289038.3275	190567.6652	51.6030	-3.6036

42	Breconshire	300222.6627	231942.1155	51.9770	-3.4541
43	Radnorshire	310673.9774	264438.2607	52.2708	-3.3105
44	Carmarthenshire	250916.8054	224617.5287	51.9001	-4.1683
45	Pembrokeshire	201091.5282	221153.1974	51.8534	-4.8896
46	Cardiganshire	258517.9060	263912.5585	52.2551	-4.0742
47	Montgomeryshire	303004.5795	302562.9454	52.6121	-3.4339
48	Merionethshire	278561.2881	327272.6737	52.8292	-3.8039
49	Caernarvonshire	255533.1773	353047.4757	53.0551	-4.1570
50	Denbighshire	309254.3430	353290.1383	53.0691	-3.3558
51	Flintshire	319181.4298	370393.2222	53.2244	-3.2120
52	Anglesey	241857.9332	379161.2517	53.2858	-4.3737
53	South Lincolnshire	512501.1099	337177.6993	52.9202	-0.3282
54	North Lincolnshire	517464.7911	386346.6489	53.3609	-0.2364
55	Leicestershire	464516.1949	308055.9699	52.6664	-1.0474
56	Nottinghamshire	466217.9433	359631.3445	53.1297	-1.0118
57	Derbyshire	426710.7915	359698.2888	53.1338	-1.6022
58	Cheshire	365211.0409	371128.8090	53.2360	-2.5227
59	South Lancashire	364026.2758	411756.4408	53.6011	-2.5451
60	West Lancashire	349899.3699	450582.9496	53.9489	-2.7649
61	South-east Yorkshire	497775.5351	447408.7594	53.9136	-0.5129
62	North-east Yorkshire	465976.2723	491112.9891	54.3114	-0.9874
63	South-west Yorkshire	432299.0538	413053.0639	53.6130	-1.5133
64	Mid-west Yorkshire	413295.4665	455532.2418	53.9957	-1.7987
65	North-west Yorkshire	404873.2946	496806.8933	54.3667	-1.9265
66	County Durham	419869.7416	536985.5684	54.7275	-1.6930
67	South Northumberland	393900.4599	577801.6620	55.0946	-2.0971
68	North Northumberland	403150.4835	623703.5561	55.5071	-1.9517
69	Westmorland	350685.3000	500008.2746	54.3931	-2.7610
70	Cumberland	333121.2858	537213.5092	54.7254	-3.0399

Appendix 2

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Validation of biological collections as a source of phenological data for use in climate change studies: a case study with the orchid *Ophrys sphegodes*

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Summary

1. The scarcity of reliable long-term phenological data has severely hindered the study of the responses of species to climate change. Biological collections in herbaria and museums are potential sources of long-term data for such study, but their use for this purpose needs independent validation. Here we report a rigorous test of the validity of using herbarium specimens for phenological studies, by comparing relationships between climate and time of peak flowering derived from herbarium records and from direct field-based observations, for the terrestrial orchid *Ophrys sphegodes*.
2. We examined herbarium specimens of *O. sphegodes* collected between 1848 and 1958, and recorded peak flowering time directly in one population of *O. sphegodes* between 1975 and 2006. The response of flowering time to variation in mean spring temperature (March–May) was virtually identical in both sets of data, even though they covered different periods of time which differ in extent of anthropogenic temperature change. In both cases flowering was advanced by *c*. 6 days per °C rise in average spring temperature.
3. The proportion of variation in flowering time explained by spring temperature was lower in the herbarium record than in direct field observations. It is likely that some of the additional variation was due to geographical variation in collection site, as flowering was significantly earlier at more westerly sites, which have had warmer springs, over their range of 3.44° of longitude.
4. Predictions of peak flowering time based on the herbarium data corresponded closely with observed peak flowering times in the field, indicating that flowering response to temperature had not altered between the two separate periods over which the herbarium and field data were collected.
5. *Synthesis*. These results provide the first direct validation of the use of herbarium collections to examine the relationships between phenology and climate when field-based observational data are not available.

Key-words: biological collections, climate change, flowering time, herbarium specimens, natural history collections, *Ophrys sphegodes,* Orchidaceae, phenology, spring, temperature

Introduction

Phenological events respond directly to climate. Recent climate change has undoubtedly affected the timing of development and seasonal events in many groups of organisms, including amphibians (Beebee 1995), birds (Crick *et al.* 1997), fungi (Kauserud *et al.* 2008) and plants (Sparks & Carey

1995; Fitter & Fitter 2002). Understanding the effects of recent climate change is a vital step towards predicting the consequences of future change. Moreover, only by elucidating the responses of individual species will we be able to predict the potentially disruptive effects of accelerating climate change on species interactions.

Detecting phenological trends in relation to long-term climate change is not straightforward. Because trends can be concealed by short-term inter-annual climate variation

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(Badeck *et al.* 2004), long datasets are needed. For most species, data collected specifically for the study of climate-induced phenological change are not available, or are difficult to find, reflecting the scarcity of long-term monitoring schemes (Sparks & Carey 1995). The choice of species for long-term studies of phenology has thus been dictated up to now by the availability of suitable field records. A further major obstacle is that most long-term data only record the beginning of phenological events in populations, such as dates of first flowering. Miller-Rushing, Inouye & Primack (2008) have shown that the use of such data to infer changes in phenology can be unreliable, and they advise that dates on which phenological stages reach their peak are preferable. However, long-term field-based records of the dates on which phenological events are at their peak are extremely rare.

Specimen-based records in biological collections are another potential source of data, verifiable in both space and time, for the study of climate-induced phenological change. Until recently, the potential of such records has been largely overlooked (Suarez & Tsutsui 2004), even though the only data available for studying phenological trends in many species are those held in natural history collections in museums or herbaria. Recent phenological studies have utilised less orthodox data sources, including historical archives (Aono & Kazui 2008), photographs (Miller-Rushing *et al.* 2006; Sparks, Huber & Croxton 2006; Crimmins & Crimmins 2008) and herbarium specimens (Primack *et al.* 2004; Bolmgren & Lönnberg 2005; Lavoie & Lachance 2006; Miller-Rushing *et al.* 2006; Bowers 2007; Kauserud *et al.* 2008; Gallagher, Hughes & Leishman 2009). Herbarium records are unique amongst these sources of information in that they capture an individual plant's phenological state at the time and location of collection, and therefore may represent a substitute for field observation. Herbarium specimens are also likely to have been collected when phenological stages such as flowering are near their peak, rather than at an early or late stage in such seasonal events. Recent studies suggest that herbarium collections may provide data that can be exploited in climate change studies, because findings have been broadly in line with trends reported in the phenological literature (Sparks 2007) and have supported the predictions of physiological models of phenological events such as flowering (Bowers 2007). Nevertheless, they depend on averaging-out the numerous possible influences and biases involved in a collection process that was not designed with the study of phenology in mind, within which the climatic signal-to-noise ratio might be low. Given the absence of long-term monitoring for most species, there is little direct evidence from which to evaluate the potential of averaged trends in events such as flowering time, derived from herbarium collections, as proxies for field data.

We report a critical comparison of independent field- and herbarium-derived data as predictors of flowering time in a species (the terrestrial orchid *Ophrys sphegodes*) for which a unique long-term phenological record of peak flowering time was available (Hutchings 2010). As the flowering time of plants that flower in early summer is generally advanced after warmer springs, we examined relationships between the flowering date of *O. sphegodes* and climate in the 9 months prior to flowering. This corresponds with the period from the end of tuber dormancy to flowering in this species. Specific hypotheses were (i) that flowering date would be advanced by warmer springs, (ii) that the relationship between flowering date and mean spring temperature would be the same in data derived from herbarium records and annual field observations, and therefore (iii) that in a particular species for which this test is possible, herbarium records would be validated both as an effective proxy for long-term monitoring in climate change research and as a predictor of phenological responses to future climate change.

Materials and methods

STUDY SPECIES

Ophrys sphegodes (the early spider orchid) is a species of southern and central Europe, with a northern range limit that includes southern England. It is associated with ancient, species-rich grassland over calcareous soils. At present the species is rare in the UK, where it is largely confined to Dorset, West and East Sussex and Kent (Lang 1989; Harrap & Harrap 2005).

Although the length of the mycotrophic, subterranean phase of the life cycle of *O. sphegodes* is unclear, it is a short-lived species after its first appearance above ground, rarely flowering for more than three consecutive years. Few plants survive for more than 10 years after initial emergence (Hutchings 1987, 2010) and most survive for less than 3 years. In the UK, the leaves of *O. sphegodes* emerge above ground in September or October (Hutchings 1989). The flowering period is relatively short, commencing during late April or early May, and usually ending by late May (Lang 1989). In most populations in the UK inflorescences bear from one to six flowers (usually two or three), which open in succession from the bottom of the inflorescence. Pollination is followed by rapid withering of the flower. Sanger & Waite (1998) found that the number of inflorescences bearing ripening seed peaked at the end of June and that rapid dieback of the plant ensued; few plants remain above ground at the end of July. This relatively short reproductive period would be expected to conserve any climatically-induced phenological signal.

HERBARIUM DATA

We examined all 192 specimens of *O. sphegodes* held in herbaria at the Natural History Museum, London (BM, 133 specimens) and Royal Botanic Gardens, Kew (K, 59 specimens) to verify identification. All of the specimens originated from southern coastal counties of England (Dorset, Isle of Wight, Hampshire, East and West Sussex, and Kent), reflecting the limited historical distribution of *O. sphegodes* (Carey & Dines 2002). The geographical range of the sites from which specimens were collected was 3.44° (decimal) longitude and 0.76° (decimal) latitude. Specimens with incomplete data for site of collection and collection date were discarded. Because of the rarity of *O. sphegodes* in the UK, the dataset was comparatively small and therefore it was important to ensure that the records represented the peak flowering stage as closely as possible. For this reason only specimens with at least 60% of their flowers open were included in the study; normally most of the flowers are open at the same time in *O. sphegodes*. Some of the herbarium sheets consisted of multiple specimens mounted together. As the specimens in such cases had been collected by a single collector, on the same day and at the same location, they were treated as non-independent and the mean percentage

of open flowers was derived. Individuals in fruit or with senescent flowers were excluded.

We rejected 53% of the 192 specimens: 2 were damaged, 9 had unclear or illegible records of collection date, 31 were not dated, 60 were imprecisely dated (only the month or year), 3 were in seed, and 1 presented fewer than 60% of flowers open. Nine specimens were duplicates (multiple specimens) and therefore mean results were used. The final data set comprised 77 specimens providing at least one data point for each of 57 years, spanning a 111-year period from 1848 to 1958.

FIELD DATA

Records of the peak flowering time of *O. sphegodes* were made in 25 of the 32 years between 1975 and 2006 in a demographic study of a population consisting of many thousands of plants at Castle Hill National Nature Reserve, East Sussex, UK (Hutchings 2010). Peak flowering was based on assessment of the entire population to give a central tendency that would fit the flowering phenology of as many individual plants as closely as possible.

METEOROLOGICAL DATA

Mean monthly Central England Temperature (CET) records for the period 1848–2006 (Parker, Legg & Folland 1992) were obtained from the UK Meteorological Office (http://hadobs.metoffice.com/hadcet/cetml1659on.dat). This is the only complete climate record available for the years during which the herbarium records and field data were collected. However, data for Central England are strictly representative only for a roughly triangular area enclosed by Bristol, Preston and London (Parker, Legg & Folland 1992). This is to the north of the distribution range of *O. sphegodes*. Monthly mean temperatures were available from two Meteorological Office weather stations on the south coast, in locations corresponding with eastern and western centres of the distribution of *O. sphegodes*. Eastbourne, East Sussex UK, 21 km east of the Castle Hill field site, operated for the period during which the field records were collected. Monthly minimum and maximum temperature data were available for Southampton, to the west, for all but 5 of the 111 years of the collection period covered by the herbarium specimens. Data from both of these collection sites would be expected to represent the climate within the distribution range of *O. sphegodes* better than the climate records available from CET. The means of monthly minimum and maximum temperature were used for both stations. Historical temperature data were not available closer to any of the sites of collection of the individual specimens in the herbarium records.

ANALYSIS

The distribution of collection dates in the herbarium dataset for 1848–1958, expressed as number of days after 1 April, was checked for normality and presence of outliers. The peak flowering date for the Castle Hill population in the years 1975–2006 was similarly expressed as days after 1 April.

Both sets of flowering phenology data were examined for relationships with mean CET temperature data from the 9 months prior to the flowering season (i.e. the period of growth following breaking of tuber dormancy the previous summer). These data included mean monthly temperature and its averages over successive 3-month periods (September–November, December–February, and March–May). This was carried out to establish which temperature variables had the highest predictive power for flowering time in both sets of

Table 1. Comparison of correlations between flowering date and temperature for the herbarium records and the field data. Correlations are shown with mean temperatures for 3-monthly periods and individual months in the same year as flowering (January–May) or in the year previous to flowering (September–December). A negative correlation indicates that a higher mean temperature is associated with an earlier flowering date

Period of mean temperature	Herbarium data (1848–1958) $n = 77$	Field data (1975–2006) $n = 25$
Seasons:		
September–November	−0.004	−0.072
December–February	−0.065	−0.610**
March–May	−0.426**	−0.801**
Months:		
September	0.008	−0.273
October	0.108	0.226
November	−0.106	−0.171
December	0.047	−0.085
January	−0.003	−0.579**
February	−0.159	−0.549**
March	−0.396**	−0.609**
April	−0.153	−0.405*
May	−0.259*	−0.592**

**$P < 0.01$; *$P < 0.05$.

phenological data. Multiple regressions using mean temperatures for the individual months failed to produce a single model that could be applied to both of the datasets, because of collinearity between the variables, and the fact that the models included different individual months for the two data sets. However, the mean temperature for the 3 months from March to May had the highest individual correlation with peak flowering date in both sets of data in an analysis of single variables (Table 1). This was designated 'mean spring temperature' and was adopted as the single predictor variable in comparisons of the phenological responses in herbarium and field data. Models using mean spring temperature accounted for only marginally less variation than the best combinations of months in separate stepwise (forward) multiple regressions. In order to investigate whether distance from the weather station influenced the relationship, the phenological analysis was repeated using Eastbourne mean spring temperature data for the field phenological regressions and equivalent Southampton data for the herbarium phenological regressions.

Variation in flowering time among the herbarium specimens was further investigated using a regression on (decimalised) longitude of origin. This sought to identify geographical sources of variation.

The linear regression model derived from the herbarium data and CET was used to predict peak flowering dates from mean spring temperature for the years between 1975 and 2006 for which field observations were available. Regression analyses were carried out with SPSS 16 (SPSS Inc., Chicago, IL, USA). Slopes and intercepts of regressions were compared using Graphpad Prism 5 (Graphpad software Inc., La Jolla, CA, USA). Predicted flowering dates were compared with observed flowering dates using principal axis regression (Sokal & Rohlf 1969).

Results

Data derived from herbarium specimens over the 111-year period from 1848 until 1958, and recorded in the field between

1975 and 2006, both confirmed the importance of spring temperature in determining flowering time. We found significant individual correlations between peak flowering date and several measures of mean temperature in the CET records in the preceding months (Table 1). For herbarium material, there were significant correlations with mean temperature in March and May of the year of flowering but the highest correlation was with mean temperature over the 3 month period from March–May. Results for the field data were similar, but with significant correlations for January, February, March, April and May. The strongest correlation was again with the mean for the period March–May.

As predicted, warmer years were associated with earlier flowering. The regression of flowering date obtained from the herbarium specimens on mean March–May (spring) temperature (Fig. 1a) accounted for 18% of the variation in flowering time. A 1 °C increase in mean temperature between March and May was associated with an advance in flowering of 6.5 days. Analysis of the field data yielded strikingly similar results. Linear regression of flowering date on mean spring temperature accounted for 64% of the variation in date of flowering (Fig. 1b) and a 1 °C increase in mean spring temperature was associated with an advance in flowering of 6.7 days. The regression models derived from the herbarium data and field data were statistically indistinguishable: neither the gradi-

ents ($F_{1,98} = 0.0035$, $P = 0.952$) nor the intercepts ($F_{1,99} = 0.0908$, $P = 0.764$) were significantly different, indicating that the phenological response to temperature was the same during the different periods over which the two sets of data were collected.

Applying the same analysis with less geographically distant temperature data for the field and herbarium records gave significant and strikingly similar results. Spring temperature at Southampton accounted for 13% of the phenological variation in herbarium data (Fig. 2a) and Eastbourne temperature accounted for 59% of that in the field data. In both cases flowering advanced by 5.7 days per 1 °C increase in spring temperature. The two regressions were again statistically indistinguishable (gradients, $F_{1,93} = 0.00007$, $P = 0.993$; intercepts, $F_{1,94} = 0.854$, $P = 0.358$). Furthermore, the gradients of the two regressions of field data on temperature recorded at Eastbourne and CET were not significantly different ($F_{1,46} = 0.481$, $P = 0.491$), and neither were the gradients of the two regressions with herbarium data using Southampton and CET temperature records ($F_{1,145} = 0.130$, $P = 0.719$); this indicates that the predicted flowering responses of the plants to temperature were consistent irrespective of the temperature records used. In both of these comparisons the intercepts were significantly different (field data, $F_{1,47} = 14.6$, $P = 0.004$; herbarium data, $F_{1,146} = 10.3$, $P = 0.002$),
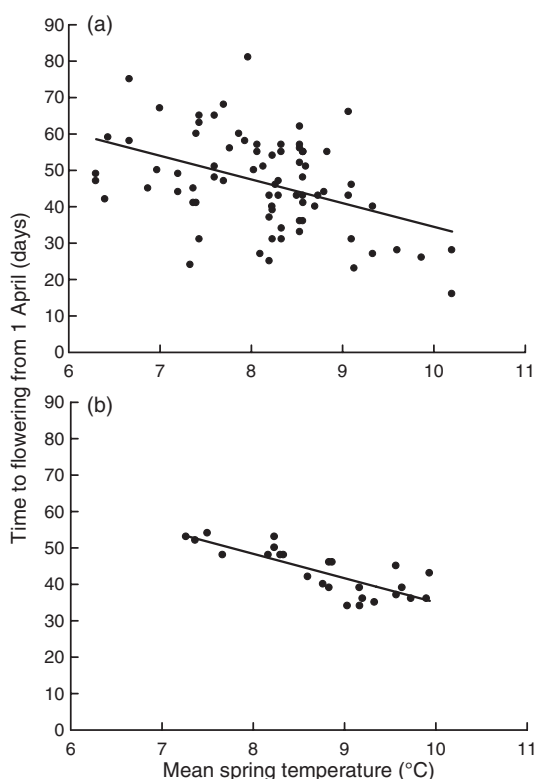

Fig. 1. Relationships between flowering date (expressed as days after 1 April) and mean spring temperature (March–May) in Central England derived from (a) herbarium records from 1848 to 1958 ($y = 99.54 - 6.51x$, $r^2 = 0.182$, $P < 0.001$, $n = 77$) and (b) field data between 1975 and 2006 ($y = 101.88 - 6.69x$, $r^2 = 0.642$, $P < 0.0001$, $n = 25$).
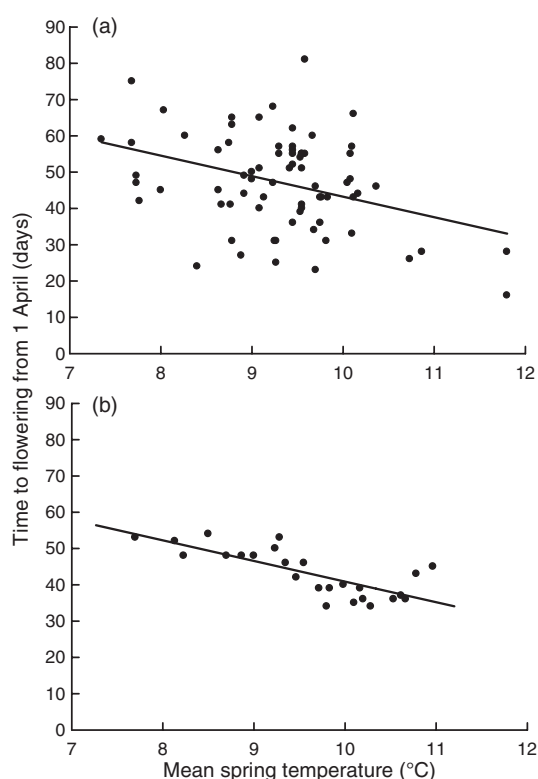
Fig. 2. Relationships between flowering date (expressed as days after 1 April) and mean spring temperature (March–May): (a) between herbarium records from 1855 to 1958 and temperature at Southampton ($y = 99.8 - 5.66x$, $r^2 = 0.134$, $P = 0.0016$, $n = 72$); (b) between field data from 1975 to 2006 and temperature at Eastbourne ($y = 97.7 - 5.68x$, $r^2 = 0.586$, $P < 0.0001$, $n = 25$).
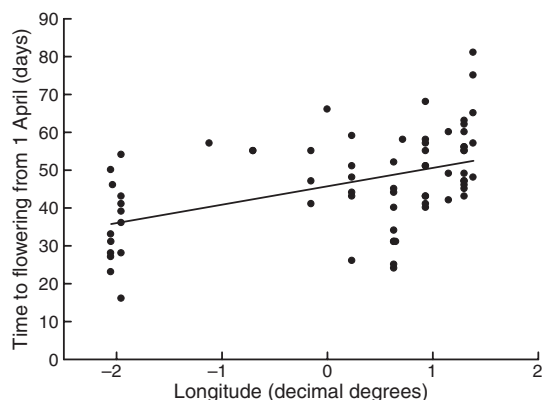
Fig. 3. Relationship between flowering date (expressed as days after 1 April) and longitude of collection site for the herbarium records. Negative values of decimalised longitude are westerly ($y = 45.74 - 4.86x$, $r^2 = 0.219$, $P = <0.001$, $n = 69$).
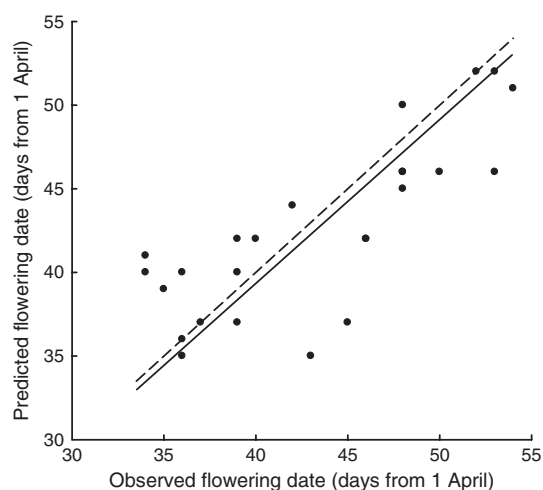

Fig. 4. Relationship between observed flowering date in the field (y_1) in 25 years between 1975 and 2006, and flowering date predicted from herbarium data for the same years (y_2). The principal axis regression (solid line) is $y_1 = -0.173 + 1.021y_2$, $r^2 = 0.63$, $P < 0.001$, $n = 25$. The dashed line would apply if there were exact correspondence between the observed flowering date and the predicted flowering date.

reflecting the differences between the temperature records used.

The effect of longitude of origin on the flowering time of herbarium specimens was significant (Fig. 3). Flowering was earlier at more westerly collection sites by an average of 4.86 days per degree longitude.

The regression model derived from herbarium specimens (1848–1958) and CET was used to predict flowering dates for each of the 25 years between 1975 and 2006 for which there were field records of time of flowering. These predictions were highly correlated with the observed peak flowering dates ($P < 0.01$); the principal axis regression between observed and predicted dates had a coefficient close to unity (1.021) and accounted for 63% of the variation (Fig. 4).

Discussion

Although biological collections can potentially provide valuable evidence of the impacts of climate change on the phenology of plant and animal species (Sparks 2007), their value as a proxy for field data has not previously been tested independently for any species. Miller-Rushing *et al.* (2006) compared flowering dates in recent benchmark years with those derived from historical photographs and herbarium specimens (1900–1921) for a range of species and found that not only were the deviations highly correlated with the corresponding differences in spring temperature but they yielded a trend that was very similar to that observed in independent field data of first flowering dates for the years 1887–1903. Bolmgren & Lönnberg (2005) established correspondence between flowering times derived from herbarium records and phenological observations, but did not investigate the underlying climatic drivers. The power of historical collection data to predict the consequences of future climate change needs to be tested directly. The availability of field data for the rare terrestrial orchid *Ophrys sphegodes*, recorded at a single site in the UK over a 32-year period, provided a unique opportunity to seek validation of the relationship between flowering date and mean spring temperature that was apparent from analysis of data from herbarium specimens collected over a much longer, and different, period of years. The comparison is greatly strengthened by the fact that peak flowering time was recorded in the field, rather than date of first flowering, which is more common in long-term phenological records. It is now clear that first flowering dates may not be ideal measures of plant responses to climate change, because the extremes of flowering distributions are more susceptible to confounding effects than central values (Miller-Rushing, Inouye & Primack 2008). Herbarium collections also tend to reflect peak flowering, as collectors generally aim to obtain prime specimens in full flower, as testified by the fact that we had to discard only one specimen in which too few flowers were open to satisfy our sampling criterion.

Both historical and contemporary data showed that the peak flowering date of *O. sphegodes* was earlier in years with warmer springs, as expected (see also Hutchings 2010). This was the case both when the two phenological records were related to a common temperature record (CET) and when field and herbarium records were related to different but more geographically proximate temperature records (Eastbourne and Southampton respectively). The close correspondence between field and herbarium regressions, irrespective of the geographical locations of the temperature records tested, argues for the robustness of the relationships. Furthermore, using geographically different temperature records did not significantly alter the results for either contemporary or historical sources of data. Previous phenological studies have found similar correlations between flowering date and measures of spring temperature in spring- and summer-flowering species. The estimated advance in peak flowering date of 5.7–6.7 days per 1 °C rise in temperature in *O. sphegodes* is within the range reported for advance in first flowering date in other species in the UK. Fitter *et al.* (1995) reported a mean advance of first flowering

date of 4.4 days per 1 °C for 243 species at a single locality but with considerable differences between species; similarly, first flowering dates of 24 species, averaged across the UK, advanced between 2 and 10 days per 1 °C increase in temperature (Sparks, Jeffree & Jeffree 2000).

The relationships between peak flowering date and spring temperature derived from contemporary and historical data for *O. sphegodes* were nearly identical, indicating a common response to spring temperature, notwithstanding that the historical collection and field observation periods were dissimilar in length, separated in time and different in geographical extent. This consistent response is important, as the pace of climate change has accelerated since 1975 when the field studies were initiated (IPCC 2007). None of the herbarium specimens was collected after 1958 and they therefore largely pre-date the period of fastest anthropogenic climate change. Because the field and herbarium data were independent, it was possible also to test the power of the earlier herbarium records to predict the effects of subsequent climatic warming. Importantly, although there was some variation between years in the accuracy of predictions, the overall predictive power was extremely good, with the principal axis regression line for predicted and observed values lying close to the ideal 1 : 1 relationship.

Rigorous validation of the type presented here, although only based on data for a single species to date, serves to increase confidence in the use of biological collections for predicting future phenological responses to climate change. Despite the strong underlying mean temperature signal, variation in flowering time may be influenced by a myriad of factors, and there are likely to be more confounding factors in the herbarium record than in the field data, because it includes specimens taken from a wider range of geographical locations and microhabitats. Predictions based solely on mean spring temperature in Central England accounted for 18% of the variation in flowering date seen in herbarium specimens, but 64% of variation in flowering date in the field records from a single site. Use of more local temperature records in fact accounted for slightly (but not significantly) less variation in both cases, possibly because of the use of minimum and maximum temperatures averaged on a monthly rather than daily basis. Another important explainable source of variation in flowering time in the herbarium record was the geographical range of collection sites, as seen in the significant regression on longitude. This was the major gradient in distribution, and earlier flowering at westerly sites is consistent with a climatic trend to warmer springs in the west. This suggests that, had local temperature records been available for each collection site, even more of the variation in flowering time would have been accounted for by spring temperature. Despite the lower signal-to-noise ratio in the herbarium record, the signal was the same as in the field data and it was applicable over a much longer period. Bowers (2007) used physiological models based on previously determined flowering requirements (trigger dates and heat sums above a 10 °C threshold) to predict, retrospectively, advancing flowering dates of shrubs in the Sonoran desert through the 20th century. A correlated tendency towards earlier collec-

tion dates in herbarium material over the same period supported the hypothesis that there had been a genuine response to changing climate, especially as there was no evidence that collector behaviour had changed over the period of study. However, the use of herbarium specimens assumes that they are representative samples of the population from which they are drawn. The potential for bias resulting from variation in collection effort has been voiced as a concern by previous authors (Case *et al.* 2007). Our study demonstrates both that collector bias is not a problem when the herbarium data accepted for use in scientific studies are subjected to carefully controlled selection criteria, and that it is not necessary to have hundreds of specimens in order to extract useful information about the relationships between climate and time of flowering. Although further validation using additional species with different phenologies is desirable, the extreme scarcity of suitable field observations limits opportunities for this to be achieved at the present time. As a spring-flowering plant, *O. sphegodes* falls into a group identified as having flowering phenologies that are likely to be particularly sensitive to temperatures early in the year (Fitter *et al.* 1995), although both the scale and direction of changes in phenology can be idiosyncratic and potentially influenced by additional climatic drivers (Crimmins, Crimmins & Bertelsen 2010). Species that flower later in the summer may be less sensitive to warmer temperatures, and species that reproduce in the autumn may be sensitive in the opposite direction; analysis of 34 500 dated herbarium records of autumn-fruiting of mushrooms in Scandinavia has revealed an average delay of 12.9 days since 1980, as the growing season has been extended by warming (Kauserud *et al.* 2008).

For most species of plants and animals, biological collections are the only source of long-term phenological data. It is estimated that some 2.5 billion specimens of flora and fauna are held in biological collections worldwide (Graham *et al.* 2004). The current drive toward digitisation of collections is facilitating the dissemination of the information they contain. An estimated 60 million records are already available for a wide range of taxa *via* internet information networks such as the Global Biodiversity Information Facility and HerpNET (Graham *et al.* 2004). With appropriate validation, the exploitation of this resource will have increasing relevance and value (Prather *et al.* 2004) as we seek to understand and predict the consequences of continuing climate change.

Acknowledgements

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