Pollination mode predicts phenological response to climate change in terrestrial orchids: a case study from central Europe

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Summary

1. Herbarium collections contain long-term data for a wide range of taxa and provide unique opportunities to evaluate the importance of life-history components in driving species-specific responses to climate change. In this paper, we analyse the relationships between change in flowering dates and life-history traits within a phylogenetic framework. The study is based on an extensive data set of herbarium specimens of orchids collected in Hungary between 1837 and 2009, supplemented by recent field observations (1980–2011).

2. Of the 39 taxa investigated, 31 (79%) showed apparent advancement in mean flowering time. Among these, advancement was statistically significant in nine taxa. The rest (eight taxa) showed non-significant delays in flowering. Averaging across all taxa, flowering time advanced by 3 days (3.8% of flowering period) during the last 50 years compared with the period before 1960. In taxa showing significant advancement, flowering times advanced by 7.7 days (8.6% of the flowering period). The most extreme advancement was 13.9 days.

3. Multivariate models were used to evaluate ways in which life history may affect phenological responses to climate change. Pollination mode (i.e. deceptive vs. rewarding vs. autogamous), life span (i.e. short-lived vs. long-lived), biogeographical distribution type (i.e. Mediterranean vs. non-Mediterranean) and flowering time (i.e. mean date of blooming) emerged as important factors that influence changes in flowering through time. Phylogenetic relatedness did not predict phenological response. The strongest response was observed in orchids that flower relatively early in spring, exhibit an autogamous or deceptive pollination mechanism, have a long life span and possess a Mediterranean centre of distribution.

4. *Synthesis*. Our investigation demonstrates that the majority of Hungarian orchids have shifted their yearly mean flowering to earlier dates during the past 50 years. Certain life-history traits, but not phylogenetic relatedness, were found to be important in predicting climatic responsiveness in European terrestrial orchids.

Key-words: biological collections, flowering time, global change, herbarium specimens, life history, life span, Orchidaceae, phenological shift, phylogenetic control, plant–climate interactions, pollinator

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Introduction

Global climate change is now taking place at an unprecedented rate (Parmesan & Yohe 2003; Root *et al.* 2003; Schröter *et al.* 2005; IPCC 2007). The biological effects of this global change have already been documented by detailed studies of a wide variety of organisms; many investigations reported a shift in phenology as a response to climate change (e.g. Fitter & Fitter 2002; Parmesan 2006; Post *et al.* 2008; Végvári *et al.* 2010).

Shifting vegetation phenology is likely to be the main mediator of climate change for animals (Bonan 2008). Consequently, understanding the responses of primary producers to climate change is particularly important for understanding its effects on all trophic levels. On the other hand, plants are also affected by other organisms in interactions such as herbivory, parasitism and pollination. In the case of herbivory and parasitism, plants may benefit from shifting their phenology if the result is an ecological mismatch that reduces damage caused by parasites or herbivores. Conversely, any phenological mismatch between pollinators and flowering plants may significantly decrease pollination efficiency and thus reduce the reproductive success of plants (Fitter & Fitter 2002; Hegland *et al.* 2009; Bartomeus *et al.* 2011; Rafferty & Ives 2011). Indeed, some studies partially attribute the recent pollination crises – the serious decline of some insect-pollinated plants and their pollinators – to climate change (Dixon 2009; Anderson *et al.* 2011; Bartomeus *et al.* 2011). Consequently, plant–pollinator relationships should be considered in studies of plant responses to environmental change.

In addition to ecological interactions, at least three other factors are likely to explain interspecific variation in the reactions of plants to climate change. First, although most ecosystems are experiencing the effects of increasing temperatures (Walther *et al.* 2002), the amplitude of temperature change varies geographically (IPCC 2007). Biological responses to changing environmental conditions are therefore unlikely to be uniform on geographical scales, implying that species inhabiting different environments will show different phenological responses (Menzel *et al.* 2006; Askeyev *et al.* 2010). For example, spring phases have been advanced in western and central Europe but delayed in eastern Europe (Ahas *et al.* 2002). Hence, to get a better understanding of climate change, we need detailed investigations of plant phenological alteration from regions of the world that are currently understudied, such as central Europe.

Second, changes in climatic conditions are not always identical at different times of the year, even at the same location (Schwartz, Ahas & Aasa 2006; Walther 2010). Therefore, plants whose active periods occur in different times of year are expected to show contrasting climatic responses. Seasonal differences in plant reactions to warming have been observed both in historical investigations of phenology (Fitter & Fitter 2002; Sparks & Menzel 2002; Walther 2004) and in experimental warming studies (Price & Waser 1998; Dunne, Harte & Taylor 2003; Sherry *et al.* 2007). One would expect that plants flowering early in spring will respond more readily to

global change (Fitter & Fitter 2002; Sherry *et al.* 2007), because they are already adapted to more variable spring weather. This effect may be strengthened by the greater degree of warming during winter and spring compared with other parts of the year (Schwartz, Ahas & Aasa 2006).

Third, the ability of an organism to follow shifting environmental conditions is thought to depend on its life-history traits. A survey of British plants showed that annuals were more likely than perennials to advance flowering (Fitter & Fitter 2002). A possible explanation for this observation is that species with shorter life spans have shorter generation times and hence can adapt more quickly to changing conditions (e.g. Baker 1974; Jump & Peñuelas 2005). On the other hand, longer-lived plant species might show a greater degree of phenotypic plasticity that could, in principle, allow them to track environmental conditions associated with climate change more accurately compared with shorter-lived species (e.g. Hoffmann & Sgrò 2011). In summary, pollination mode, geographical distribution, timing of activities within the year and life span are likely to influence phenological responsiveness in plants.

Terrestrial European orchids are ideal for studying the effects of pollination mode on flowering phenology, because, although they are mainly self-compatible (Neiland & Wilcock 1999), they maintain diverse pollination modes: many of them are self-pollinating (including obligate and facultative inbreeders), others reward pollinators with nectar and approximately one-third of the species use various forms of floral deception (Dafni 1984; Jersáková, Johnson & Kindlmann 2006). The flowers of deceptive orchids mimic food or receptive females, luring insects to transfer pollen without substantially rewarding them. Pollination of deceptive plants differs from that of nectar-rewarding ones because their pollination is accomplished only by visits from inexperienced insects that have not yet learnt to avoid non-rewarding flowers (Schiestl 2005). Learning takes time; therefore, in deceptive orchids, the reproductive success of early inflorescences tends to be higher (Jacquemyn *et al.* 2002), and the earliest flowers of the inflorescence have a better chance of being pollinated (Vallius 2000; Jacquemyn *et al.* 2002). A further reason for investigating climatic responsiveness in orchids is that many species of this charismatic group are of key conservation importance (Jacquemyn *et al.* 2005; Kull & Hutchings 2006; Swarts & Dixon 2009). Finally, orchids have long been popular among both professional and amateur botanists, leading to the accumulation of extensive long-term herbarium collections. Such collections are especially important in analysing phenological responses of plants (Miller-Rushing *et al.* 2006; Primack & Miller-Rushing 2009; Robbirt *et al.* 2011; Panchen *et al.* 2012). The Herbarium Database of Hungarian Orchids has recently been compiled from all publicly accessible Hungarian herbaria (Molnár V. *et al.* 2012). The data set analysed in this paper is based on this large data base. It contains flowering data for 41 native orchid species, which represent 66% of the countries' current orchid flora (only very rare taxa are missing; Molnár V. 2011). Our data set spans more than 170 years and covers the entire present day territory of Hungary.

In this study, we utilize this large Hungarian data set to study the phenological responsiveness of orchids. Specifically, we focus on the following questions. First, given the overarching importance of ecological interactions, how does pollination mode influence the response of plants to climate change? Second, do characters related to the spatial distribution of species affect phenological response? Biogeographical distribution type (e.g. Mediterranean vs. non-Mediterranean) and the altitude of the preferred habitat can indicate complex life-history adaptations to contrasting climatic conditions. Therefore, different phenological response to global change by plants with different distribution type and altitude is expected. Third, do early flowering orchids show a stronger phenological response to climate change, mirroring what has already been demonstrated for a wide taxonomic range of flowering plants (Fitter & Fitter 2002; Sherry *et al.* 2007)? Fourth, how does life span predict the magnitude of phenological responses in plants? A larger potential to adapt to climate change has been postulated for short-lived plants, reflecting their faster reproductive cycle (Jump & Peñuelas 2005). Are short-lived orchids more responsive phenologically? Finally, we were also interested in the way phylogeny affects changes in flowering time because other studies (e.g. Willis *et al.* 2008; Davis *et al.* 2010) have found phylogenetic relatedness to be a crucially important predictor of responsiveness to changing climate.

Materials and methods

METEOROLOGICAL DATA

A subsidiary analysis of meteorological data was accomplished to characterize climatic change in Hungary during the studied period. Mean monthly temperature data for Hungary were obtained from the weather data base of the National Oceanic and Atmospheric Administration (NOAA; ftp://ftp.ncdc.noaa.gov/pub/data/ghcn/v2/). This data base is compiled from climate records of ten meteorological stations distributed throughout Hungary between 1841 and 2009. For each station, data cover almost the entire study period, although gaps between years are substantial for all stations but Budapest. To assess the consistency of climatic predictors, Pearson's correlation coefficients were calculated between the mean monthly temperature data from Budapest and all other stations. As the pairwise correlations between Budapest records and those from each comparable station were strong and highly significant ($r > 0.99$, $P < 0.001$ for all cases), we used only the continuous records available for the whole study period from Budapest. Heat accumulation, as measured via the sum of monthly mean temperature values between January and May, was used to characterize changes in temperature throughout the time frame of the analyses. This meteorological variable is a biologically more relevant predictor than temperature itself (Araújo & Luoto 2007). We also calculated average seasonal temperatures for spring (March–May), summer (June–August), autumn (September–November) and winter (December–February) as the means of the temperature of the given months. Climatic trend in temperature data was analysed by fitting linear regressions on average seasonal and cumulative temperatures as a function of year.

We were not able to investigate other meteorological variables, such as monthly precipitation, because the correlations between the different meteorological stations were not sufficiently strong ($r < 0.25$, $P > 0.07$ for all cases). Consequently, the only continuous set of records from Budapest characterizes precipitation levels in other parts of the country rather poorly.

PHYLOGENETIC TREE RECONSTRUCTION

We employed the widely used ribosomal internal transcribed spacer region of the nucleus (nrITS) (Baldwin *et al.* 1995) to reconstruct the phylogenetic relationships among our study species to investigate the effect of evolutionary ancestry on phenological response to climate change (Davis *et al.* 2010). Most sequences were retrieved from Gen-Bank (Table S1 in Supporting Information). When nrITS data for a given species were not available in the public data base, we either obtained sequences from R. M. Bateman (Bateman *et al.* 2003) or generated sequences from samples collected by us (laboratory protocol followed Gulyás *et al.* 2005). The new sequences were deposited in GenBank (Table S1 in Supporting Information). Altogether, we collected nrITS sequence data for all 41 orchid species studied (Table S1 in Supporting Information).

The final alignment, consisting of the 41 nrITS sequences of the analysed taxa, was performed using the CLUSTALW algorithm, as implemented in BIOEDIT v. 7.0 (Hall 1999). There were altogether 734 positions in the alignment, including several indels. The phylogenetic relationships were reconstructed using a heuristic search under the parsimony optimality criterion as implemented in PAUP v.4.0b10 (Swofford 2002). We used a topological constraint reflecting the established phylogenetic relationships (Bateman *et al.* 2003, 2005) of the species analysed (Fig. 1). This constraint was applied to avoid biased phylogenetic reconstruction due to suboptimal taxonomic representation resulting from the taxonomic gaps in the present sampling, which was geographically limited to Hungary. In other words, the constraint was used to reconstruct the already determined phylogenetic relationship between the taxa, but allowed for assignment of a specific length to each branch to more accurately represent relative degrees of molecular divergence. The heuristic search utilized 378 parsimony-informative positions, treated gaps as missing data, added the sequences randomly using 100 sequence replicates and used 'ACCTRAN' character-state optimization. We transformed the obtained rooted phylogram (Fig. S1 in Supporting Information) into an ultrametric tree by nonparametric rate smoothing (NPRS) using R8s v.1.71 (Sanderson 2003). This tree (Fig. 1) then formed the basis of all subsequent analyses. To assess the statistical robustness of the obtained tree branches, we performed 1000 bootstrap pseudo-replicates in PAUP v.4.0b10.

PHENOLOGICAL DATA

Historical data on 41 orchid taxa were gathered from the Herbarium Database of Hungarian Orchids (Molnár V. *et al.* 2012), which contains 7658 records. We used a subset of the total data available in the data base, including only specimens bearing an exact collection date (specified to a single day), validated specific identification and at least one flower. Herbarium sheets containing several specimens collected on the same day, at the same locality, belonging to the same taxa were counted as a single record. Specimens with flowers withered or in fruit were excluded. Altogether, 5424 herbarium records were included in the analyses, which spans the period 1837–2009. Since plant collecting in Hungary, as in other regions (Prather *et al.* 2004), is declining, there are fewer herbarium specimens available from recent decades. This is especially true for orchid species, most of which have been protected by law in Hungary since 1982. To balance
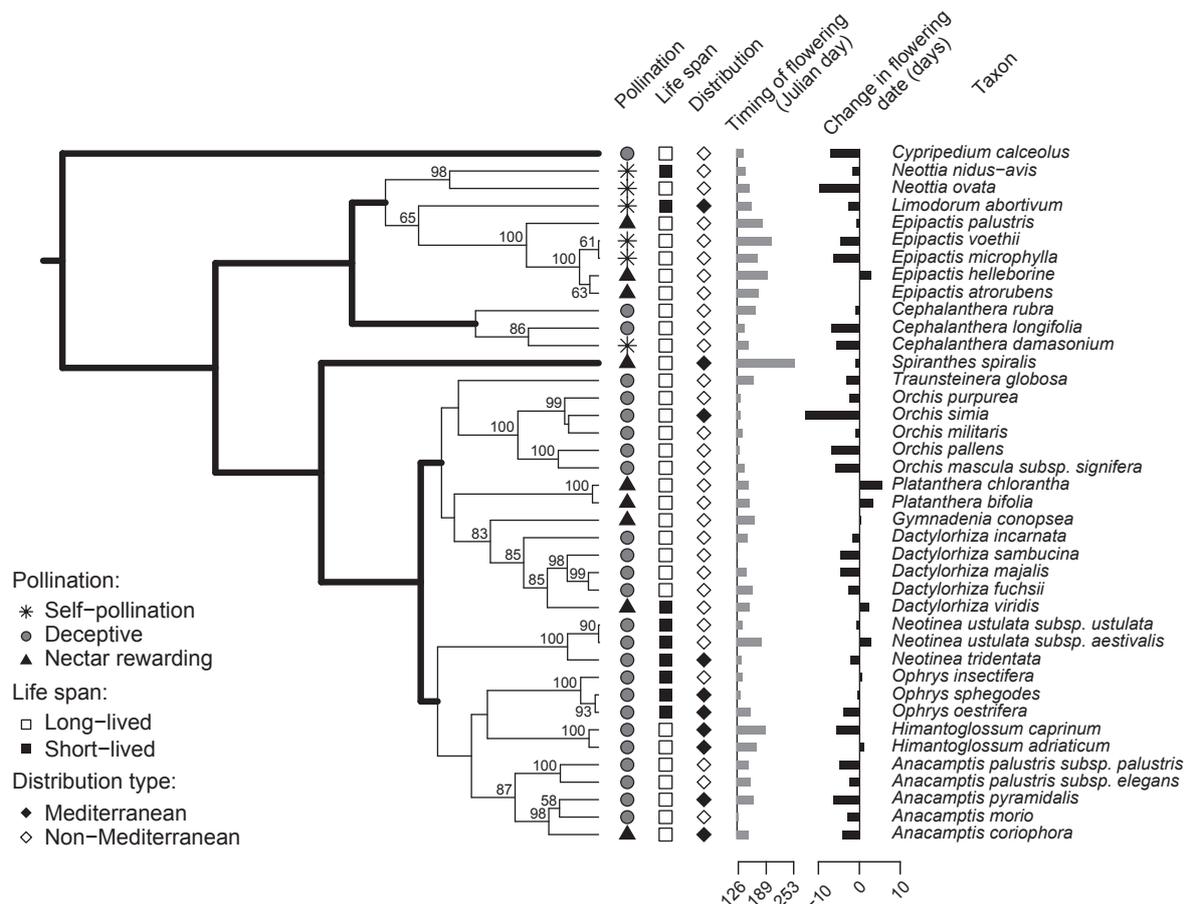
Fig. 1. Maximum parsimony ultrametric phylogenetic tree used in this study, with trait values for pollination mode, life span, distribution type, flowering time and shift in flowering date after 1960 shown for each taxa. The topological constraint enforcing the previously established relationship of main groups is drawn as thick branches, whereas the 1000 bootstrap support values are drawn next to the corresponding branches. Thin branches without bootstrap value collapse in the majority rule consensus tree.

the lack of herbarium records from recent decades, we added 2071 field observations of the 41 taxa to the data set recorded in Hungary by A.M.V. and J.S. These field-collected data cover all of the information that was retrievable from herbarium sheets, including phenological state, observation date and place, making the field data fully compatible with the herbarium records.

Assessed across all species, data on phenology were available for an average of 66 years; however, in the case of the bee orchid (*Ophrys apifera*), specimens were collected only in 8 years. We therefore removed this species from all subsequent analysis. Most species in our data set have mean flowering dates in late spring or early summer; the only exception is Autumn Lady's-tresses (*Spiranthes spiralis*), which is the latest-blooming Hungarian orchid (mean flowering date: 10 September). Because this species constitutes a very strong outlier when considering flowering time and climatic events may have different effects in autumn than in spring, we excluded *Spiranthes* from the analyses. To determine whether this decision significantly affected our results, all analyses were repeated with *S. spiralis* included (see Results). For all other taxa, samples were available from at least 21 years, up to a maximum of 126 years spanning 101–174 year periods (Table S2 in Supporting Information).

We chose yearly mean flowering to characterize flowering time instead of first flowering dates because the former is more robust in contrasts to population size and sampling effort (Miller-Rushing, Inouye & Primack 2008). This approach was necessary for this data

base since there were many occasions when only single observations were available for a species in a given year (on average, the proportion of years with more than one record per species relative to the complete time series for that species was 51%, range, 10–75%). These yearly records, however, covered a considerable time frame in all species (on average 154 years; range, 101–174 years; Table S2 in Supporting Information), and the length of the time span covered did not correlate with temporal trends ($r_s = -0.20$, $n = 39$, $P = 0.223$); thus, temporal bias in sampling effort is unlikely to influence our results. To quantify historical changes in flowering times, we employed two approaches. First, we measured temporal trends in flowering dates by calculating Spearman's rank correlation coefficients between year and yearly mean flowering dates (since these were not always normally distributed) for each species separately. The Spearman's rank correlation coefficients (i.e. the temporal trends) were subsequently used in phylogenetic analyses as a dependent variable. Nonetheless, to obtain a more robust measure of historical change in flowering date, we calculated the difference between mean flowering dates of the periods before and after 1960 for each orchid taxon and used this index as the dependent variable in subsequent analyses. The year of 1960 was chosen as the time closest to when global climate change started to be recognized (Walther *et al.* 2002). This procedure was repeated using 1950, 1970 and 1980 as alternative thresholds (Table S3 in Supporting Information). As the results of these analyses were qualitatively similar (see Results), we here

present only the analyses using 1960 as the cut-off point. We refer to this variable as shift in flowering time, which is mean flowering date after 1960 minus mean flowering date before 1960. The two indices of historical change in flowering date (the temporal trend and the shift in flowering date after 1960) gave a strong positive correlation (Spearman's rank correlation, $r_s = 0.86$, $n = 39$, $P < 0.001$).

LIFE-HISTORY TRAITS

Species were categorized into three groups according to their pollination mode – self-pollination or autogamy, nectar-rewarding entomophily and deceptive entomophily – following Neiland & Wilcock (1999) and Claessens & Kleynen (2011). We defined self-pollination to include both obligate and facultative autogamy, since the latter strategy is virtually as independent from pollinators as the former. We recorded the biogeographical distribution type of species according to the classification of Horváth *et al.* (1995) as Mediterranean (including Mediterranean, Atlantic-submediterranean, Submediterranean) and non-Mediterranean (all other types). The Herbarium Database of Hungarian Orchids (Molnár V. *et al.* 2012) was used to quantify altitudinal distribution as the proportion of specimens per taxa collected in altitudinal vegetation belts over 300 m a.s.l.. We used several published and unpublished sources to classify the life span of each species, largely following Kull & Hutchings (2006): species were classified as either short-lived (defined as those in which genet half-life is less than 3 years) or long-lived (genet half-life is more than 3 years). Species-specific flowering time was calculated from the data base by taking the mean Julian date of records available for a given species from the first part of the sampling period (before 1960). In this way, we minimized the potential bias resulting from interspecific differences in response to warming, as phenological records from the last five decades (which experienced a marked increase in temperatures; IPCC 2007) were not taken into account. A full list of all investigated species, as well as the data used in phylogenetic analyses, is given in Table S2 in Supporting Information.

STATISTICAL ANALYSES

To analyse the effects of life-history and ecological traits on plant phenological response, we used phylogenetically generalized least squares (PGLS; Martins & Hansen 1997; Pagel 1997, 1999), as implemented in the CAIC package (Orme *et al.* 2009) in the R statistical environment (R Development Core Team 2010). PGLS is an extension of generalized least squares – a multivariate method that allows for evaluating the effect of individual predictor variables after controlling for the effect of other explanatory variables in the model. In addition, PGLS models incorporate the expected covariance among species based on their phylogenetic relationships, thereby controlling for the non-independence of data points due to the shared descent of species. The covariance matrix can be modified to allow for deviations from the standard Brownian model of character evolution; this is achieved by multiplying its off-diagonal elements by the parameter λ, thereby modifying the covariation among species (Pagel 1997, 1999). Values of λ close to 1 retain the original Brownian model of character evolution, whereas values approaching 0 imply phylogenetic independence (i.e. no covariation among species due to phylogeny). The most appropriate value of λ for a given trait or model can be found by maximum-likelihood estimation (Freckleton, Harvey & Pagel 2002). We used this procedure to set λ to the value that best fits the data in all models. We also quantified phylogenetic signal in each trait separately by estimating

the maximum-likelihood value of λ using the *geiger* package in R (Harmon *et al.* 2009); statistical significance was estimated by likelihood ratio tests (i.e. by comparing the log-likelihood of the model in which λ is set to the most appropriate value to the log-likelihood of a model in which $\lambda = 0$).

To evaluate the relative importance of predictor variables in determining phenological responses, we constructed separate multivariate models for the two dependent variables (temporal trend in flowering date and shift in flowering date after 1960) with all possible combinations of the predictors without interactions (31 possible models for the five predictors) and used Akaike Information Criterion corrected for small sample sizes (AIC$_c$) to rank these models (Burnham & Anderson 2002). For each of the models in the model set, we calculated AIC$_c$ differences (Δ_i; the difference in AIC$_c$ value between the focal model and the best model) to evaluate the level of empirical support for a given model and Akaike weights (w_i) to approximate the strength of evidence in favour of the focal model relative to the others (Burnham & Anderson 2002). Akaike differences in the range between 0 and 2 indicate substantial level of empirical support of a given model, whereas $\Delta_i > 7$ imply very low support. Because no model received decisive support, but a subset of models emerged as having substantial support, we used model averaging, and for each predictor variable, we calculated Akaike sums ($\sum$): the sum of Akaike weights of all models in which that given predictor occurs. We also calculated model-averaged parameters ($\bar{\beta}$) and unconditional standard errors (SE$_u$; Burnham & Anderson 2002); these are parameter estimates that incorporate model selection uncertainty by calculating average parameter values (and their standard errors) across all models in the model set in which the parameter appeared, weighted by the Akaike weights of these models. This implies that models receiving less support (i.e. lower Akaike weights) contribute proportionally less to the final estimates (Burnham & Anderson 2002). Confidence intervals of 95% were calculated as $\bar{\beta} \pm 1.96 SE_u$.

A potential source of bias in our estimates of phenological change is that records were collected at different locations in a relatively large geographical region. Since phenology can depend strongly on altitude and latitude, a potential geographical bias in sampling can lead to erroneous estimates of phenological change. To explore whether the location of the records affects our estimates of phenological change and the importance of life-history variables in determining this response, we first evaluated the relationships between flowering date and the altitude or the latitude of the location of the records, respectively. Since herbarium sheets give only verbal information on the location of the samples, we had to use the coordinates of the locality where a given orchid was collected. This necessarily involved some uncertainty, especially in altitude, since, for example, orchids collected on a hillside will often be assigned to the lower elevations of an adjacent village. However, given that a substantial part of these herbarium sheets are more than a hundred years old, it is impossible to obtain more accurate information on their exact collection site. Note, however, that we did not include records that could not be accurately georeferenced, for instance, those referring to large cities as collection locality (e.g. Budapest) or mountain ranges, unless further information was given on the exact location. Eventually, 5041 of the 5424 herbarium records could be georeferenced in this way. There was no such difficulty with recent data, as most of these were recorded with GPS coordinates.

Linear regressions between the Julian day of individual records as a dependent variable and elevation or latitude as explanatory variables indicated that elevation significantly affects phenology in 24 of 39 species, whereas latitude had significant effects in 15 of 39 spe-

cies. When both factors were included in a single model, elevation had a significant effect in 20 species, whereas latitude affected only six species. This suggests that elevation is generally more important than latitude on our geographical scale, but both factors affect phenology in some species and neither of them in other species. Therefore, to obtain estimates of phenological change with the effects of geographical differences removed, we first had to evaluate which of the two factors affects phenology and then calculate residuals from models containing the significant parameters only. To this end, we fitted four general linear models for each species: the first (M1) contained elevation, latitude and their interaction (since it is conceivable that elevation could have a different effect at lower latitudes than at more northern sites); the second (M2) contained elevation and latitude, but not their interaction; the third (M3) contained elevation only and the fourth (M4) was the null model (i.e. it contained only the intercept). Since these are nested models (M1⊃M2⊃M3⊃M4), they can be compared by means of an *F*-test. We sequentially compared M1 with M2, M2 with M3 and M3 with M4 and accepted the simpler model if they did not differ significantly. The simplest model that did not differ statistically from the more complex models was selected as the best for that particular species; in this way, M1 was identified as the best model in two species, M2 in seven species, M3 in 15 species and M4 in 15 species. Residuals were extracted from the best model, and their yearly means calculated; these were subsequently used to calculate temporal trends and shift in flowering dates. The estimates of phenological change obtained in this way correlated very strongly with the estimates obtained from the raw data (r_S = 0.95 and 0.93, respectively; n = 39, $P < 0.001$) in both cases, suggesting that our measures of phenological change based on the raw data are not biased. Therefore, the results presented below are based on the raw data.

Results

CLIMATIC TRENDS

We detected a significant overall increase in January–May cumulative temperatures between 1841 and 2009 (F = 4.763; R^2 = 0.028, b = 0.021, P = 0.031, d.f. = 1,168). This measure of temperature accumulation averaged for the years after 1960 was 1.749 °C higher than in the previous part of the study period. Winter was the only season with significantly increasing temperatures (F = 7.996, R^2 = 0.045, b = 0.008, P = 0.05, d.f. = 1,168). Monthly mean temperatures increased only in February (F = 4.527, R^2 = 0.026, b = 0.009, P = 0.035, d.f. = 1, 168) and March (F = 6.565, R^2 = 0.038, b = 0.009, P = 0.011, d.f. = 1,168).

PHYLOGENETIC TREE

The maximum parsimony search with the topological constraint yielded two equally most-parsimonious trees (length = 1466 steps, CI = 0.597, RI = 0.823) that differed only in the relative placements of two sister species, *Orchis simia* and *Orchis purpurea* – species that have experienced extensive and recent gene flow (Bateman, Smith & Fay 2008). Given the trivial nature of this topological difference, only one topology was used in further analyses (Fig. 1). The relationships of terminal taxa were broadly congruent with

those published for the corresponding subfamilies by Bateman *et al.* (2003, 2005). Apparent differences were found in three cases: (i) the placement of *Transteinera* relative to the genus *Orchis*; (ii) placement of *Himantoglossum* relative to the genus *Anacamptis*; (iii) placement of *Anacamptis pyramidalis* relative to *Anacamptis morio* and *Anacamptis coriophora*. All these placements reflect branches that are statistically unsupported on both our tree and the previously published trees.

HISTORICAL CHANGES IN FLOWERING TIME

Of the 39 taxa analysed (after *O. apifera* and *S. spiralis* were excluded), flowering time advanced in 31 cases; in nine of them, the shift differed significantly from 0 (Table S2 in Supporting Information). Although the remaining eight species showed delay in flowering date, none of these shifts was statistically significant (Table S2 in Supporting Information). On average, flowering dates during the last 50 years occurred 3 days earlier than during the period before 1960 (assessed across all taxa). Compared with the range of flowering dates before 1960 (i.e. the taxon-specific length of the blooming period), this amounts to a 3.8% advancement. However, the shift in flowering dates differed strongly among species, ranging from an advancement of 13.9 days (12.7%) in *O. simia* to a delay of 5.8 days (8.6%) in *Platanthera chlorantha* (Fig. 1 and Table S2 in Supporting Information).

Phylogenetic signal (λ) was zero for both measures of historical change in flowering date; significant phylogenetic signals were, however, detected in flowering time, pollination mode and life span (Table 1).

Multivariate analysis of the factors affecting interspecific variation in the two dependent variables (the temporal trend and the shift in flowering date after 1960) and subsequent model selection revealed that strongly supported models (i.e. those with $\Delta_i < 2$; Burnham & Anderson 2002) contained two predictors in both cases: pollination mode and life span (Table 2). Nonetheless, mean flowering time and geographical distribution were also found to be important predictors, but only in characterizing either temporal trends in flowering date (the former) or shift in flowering time (the latter). After model averaging, the most important factor explaining temporal trends in flowering date is pollination mode (Σ = 0.975; Table 3): advancement

Table 1. Lambda statistics for phylogenetic signal in the investigated response (first two rows) and explanatory variables

	λ	Likelihood ratio	*P*
Temporal trend in flowering	0	< 0.001	NS
Shift in flowering date after 1960	0	< 0.001	NS
Flowering time	0.56	6.85	0.009
Pollination mode	1	16.35	< 0.001
Geographical distribution	0.62	0.28	NS
Life span	1	13.65	< 0.001
Altitudinal distribution	0.65	2.27	NS

Significant lambda indicates that the value of the given variable is significantly affected by phylogenetic relatedness. For details of the analyses, see main text.

Table 2. Comparison of multivariate phylogenetically generalized least squares (PGLS) models for historical changes in flowering dates based on Akaike Information Criterion corrected for small sample sizes (AIC_c). AIC_c values, along with the number of parameters (k; including the intercept and the residual error estimate), AIC_c differences (Δ_i), Akaike weights (w_i) and adjusted R^2 (R^2_{adj}) are presented for each model. Models with $\Delta_i < 2$ are considered to have substantial support, whereas Akaike weights provide an estimate of the probability that the given model is actually the best in the model set. Only models with $\Delta_i < 7$ are shown

Predictors		k	AIC_c	Δ_i	w_i	R^2_{adj}
Temporal trend in flowering date						
0	FT+POL+LS	6	−45.837	0.000	0.422	0.479
1	FT+POL+DIST+LS	7	−45.638	0.199	0.382	0.525
2	FT+POL+LS+ALT	7	−42.033	3.804	0.063	0.468
3	FT+POL+DIST+LS+ALT	8	−41.390	4.447	0.046	0.512
4	FT+POL	5	−39.552	6.285	0.018	0.355
5	POL+LS	5	−39.431	6.406	0.017	0.340
Shift in flowering date after 1960						
0	FT+POL+DIST+LS	7	206.585	0.000	0.400	0.535
1	POL+DIST+LS	6	207.104	0.519	0.308	0.491
2	POL+LS	5	209.255	2.670	0.105	0.422
3	FT+POL+LS	6	210.431	3.846	0.058	0.446
4	FT+POL+DIST+LS+ALT	8	210.774	4.189	0.049	0.523
5	POL+DIST+LS+ALT	7	211.285	4.700	0.038	0.475
6	POL+LS+ALT	6	212.372	5.787	0.022	0.417

FT, flowering time; POL, pollination mode; DIST, geographical distribution type; LS, life span; ALT, altitudinal distribution.

Table 3. Relative importance of explanatory variables in determining phenological responses of Hungarian orchids as shown by their Akaike sums ($\sum$)

Relative importance	Temporal trend in flowering date	Shift in flowering date after 1960
1	POL ($\sum = 0.975$)	POL ($\sum = 0.999$)
2	FT ($\sum = 0.966$)	LS ($\sum = 0.992$)
3	LS ($\sum = 0.958$)	DIST ($\sum = 0.797$)
4	DIST ($\sum = 0.454$)	FT ($\sum = 0.519$)
5	ALT ($\sum = 0.122$)	ALT ($\sum = 0.121$)

FT, flowering time; POL, pollination mode; DIST, geographical distribution type; LS, life span; ALT, altitudinal distribution.

was most pronounced in autogamous orchids; taxa with deceptive entomophily showed a similar, but somewhat weaker response, whereas advancement was much weaker in nectar-rewarding taxa (Table 4). The second most important factor was mean flowering date ($\sum = 0.966$); early-blooming orchids advanced their flowering dates more than late-flowering species (Table 4). Life span also had a strong impact on temporal trends in flowering dates ($\sum = 0.958$); short-lived species showed a much weaker response than long-lived species (Table 4). Finally, temporal trends in flowering were more pronounced in taxa with a Mediterranean distribution type ($\sum = 0.454$; Table 4). By contrast, altitudinal distribution was far less important in explaining interspecific differences in phenological responsiveness ($\sum = 0.122$).

Relationships between the shift in flowering dates and the four predictors showed a somewhat different pattern (Tables 3 and 4, Fig. 2); the most important factor explaining the shift in flowering date after 1960 was pollination mode ($\sum = 0.999$), followed by life span ($\sum = 0.992$), geographical

distribution type ($\sum = 0.797$) and flowering time ($\sum = 0.519$). Again, altitudinal distribution appeared to be far less important ($\sum = 0.121$).

Covariation among most life-history variables was not significant (Table S4 in Supporting Information), suggesting that each factor is important in and of itself and that no single hidden factor is driving the results. Only Mediterranean species were found at lower elevations than non-Mediterranean taxa (18% of records originating from locations higher than 300m a.s.l in the former, 40% in the latter), and short life span appeared to be more common in Mediterranean species (four of nine Mediterranean species vs. five of 30 non-Mediterranean species were categorized as short-lived).

ADDITIONAL ANALYSIS

We checked whether the effects of predictor variables on the change in flowering time is consistent by calculating the shift in flowering date after 1950, 1970 and 1980, respectively. In all three cases, model averaging identified pollination mode as the most important factor explaining interspecific differences in the advancement of flowering, whereas the effect of the other factors was more variable between cut-off point dates (Table S3 in Supporting Information). Inclusion of the late-blooming outlier *S. spiralis* had a negligible effect on the results, slightly increasing model selection uncertainty in the case of the temporal trend analyses (Table S5 in Supporting Information).

Using residuals from the linear models employed to control for elevation and latitude to obtain estimates of phenological change yielded qualitatively similar results to the analyses based on the raw records (Table S6a and b in Supporting Information), with one notable difference: pollination mode

Table 4. Model-averaged parameter estimates for the effect of ecological and life-history variables predicting temporal trends in flowering dates. The intercept indicates long-lived, autogamous orchids with Mediterranean distribution type. Weighted averages for parameter estimates ($\bar{\beta}$), standard errors (SE$_u$) and 95% confidence intervals are shown (in italics), along with parameter estimates (±SE) for the supported models (i.e. models with $\Delta_i < 7$)

|	Intercept	Flowering time	Pollination (deceptive)	Pollination (nectar rewarding)	Geographical distribution type	Life span	Altitudinal distribution type
Temporal trend in flowering date							
0	−0.819 (0.194)	0.004 (0.001)	0.124 (0.054)	0.269 (0.062)		0.139 (0.044)	
1	−1.002 (0.197)	0.004 (0.001)	0.086 (0.061)	0.225 (0.062)	0.092 (0.043)	0.159 (0.045)	
2	−0.826 (0.196)	0.003 (0.001)	0.127 (0.055)	0.27 (0.062)		0.138 (0.045)	0.044 (0.079)
3	−1.014 (0.202)	0.004 (0.062)	0.084 (0.063)	0.222 (0.047)	0.1 (0.047)	0.162 (0.084)	−0.032 (0.001)
4	−0.727 (0.212)	0.003 (0.001)	0.056 (0.069)	0.205 (0.071)			
5	−0.225 (0.054)		0.068 (0.058)	0.269 (0.069)		0.115 (0.049)	
$\bar{\beta}$ (SE$_u$)	*−0.879 (0.248)*	*0.004 (0.001)*	*0.104 (0.062)*	*0.247 (0.067)*	*0.092 (0.044)*	*0.147 (0.046)*	*0.016 (0.091)*
95% CI	*(−1.365; −0.394)*	*(0.002; 0.006)*	*(−0.018; 0.225)*	*(0.117; 0.378)*	*(0.005; 0.18)*	*(0.056; 0.238)*	*(−0.164; 0.195)*
Shift in flowering date after 1960							
0	−18.852 (5)	0.056 (0.028)	3.4 (1.348)	7.471 (1.509)	3.017 (1.115)	4.961 (1.127)	
1	−9.232 (1.636)		2.481 (1.328)	7.471 (1.578)	2.742 (1.158)	4.514 (1.156)	
2	−6.696 (1.318)		2.1 (1.405)	7.429 (1.682)		3.76 (1.184)	
3	−14.539 (5.173)	0.047 (0.03)	2.838 (1.454)	7.424 (1.647)		4.071 (1.176)	
4	−19.054 (5.086)	0.058 (0.028)	3.396 (1.366)	7.447 (1.529)	3.253 (1.254)	5.053 (1.161)	−0.934 (2.15)
5	−9.202 (1.709)		2.474 (1.352)	7.467 (1.604)	2.781 (1.292)	4.527 (1.187)	−0.165 (2.22)
6	−7.432 (1.579)		2.239 (1.42)	7.481 (1.69)		3.734 (1.189)	1.819 (2.128)
$\bar{\beta}$ (SE$_u$)	*−13.604 (6.312)*	*0.055 (0.028)*	*2.868 (1.454)*	*7.455 (1.574)*	*2.914 (1.161)*	*4.592 (1.226)*	*0.043 (2.428)*
95% CI	*(−25.974; −1.233)*	*(−0.001; 0.111)*	*(0.018; 5.717)*	*(4.371; 10.54)*	*(0.638; 5.189)*	*(2.189; 6.995)*	*(−4.716; 4.801)*
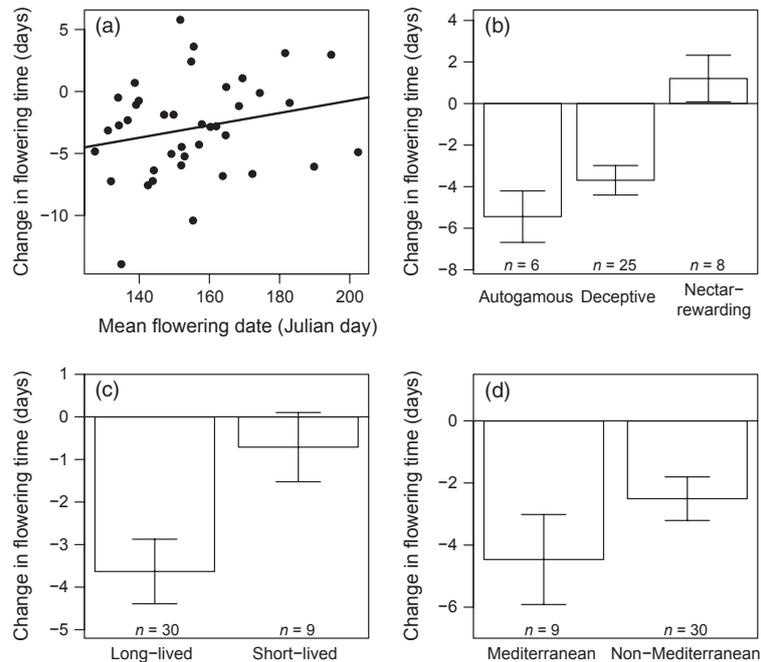
Fig. 2. The relationship between the shift in flowering date after 1960 and flowering time (a), pollination mode (b), life span (c) and geographical distribution type (d) in Hungarian orchids.

received a much lower importance ($\Sigma = 0.27$) in the temporal trend analysis, although the difference between nectar-rewarding and autogamous taxa still differed from 0 (Table S6b in Supporting Information).

Discussion

Our study yielded three main results. First, the majority of orchid taxa analysed advanced their flowering date over the

studied time period. Second, pollination mode and life span emerged as factors strongly and consistently related to the degree of advancement in flowering in all analyses, regardless of the method of measuring phenological change (Table 3). Flowering time was a strong predictor of phenological response only in the analyses considering the correlation between years and flowering time (temporal trend). Additionally, biogeographical distribution type was found to be only moderately important in predicting shifts of flowering date. Third, phylogenetic relatedness exerted little constraint in all models fitted on both trends and change of flowering dates.

Interestingly, self-pollinating orchids, which are unconstrained by pollinators, have advanced their flowering date most strongly, whereas the degree of advancement in insect-pollinated taxa depended on the specific nature of their entomophily. Deceptive species showed almost as much advancement as autogamous species, whereas nectar-rewarding orchids did not respond to increasing spring temperatures. This is consistent with the hypothesis outlined in the introduction. Nectar-rewarding species are likely to be constrained in responding to climate change due to their extensive interactions with pollinators (Hegland *et al.* 2009), if pollinators advanced more slowly than orchids would. Unfortunately, no data are available to support this idea. As for deceptive species, they compete intensively for naïve pollinators (Schiestl 2005); therefore, they are expected to be very sensitive to environmental variations in order to successfully synchronize their flowering before the first appearance of their pollinators even in years when spring starts very early. Indeed, they flower earlier than nectar-rewarding orchids (Internicola, Bernasconi & Gigord 2008; Internicola & Harder 2012; Pellissier *et al.* 2010). Accordingly, mean flowering date of deceptive orchids in our sample is 30 May, while it is 15 June for nectar-rewarding species. (note that the effect of pollination mode on phenotypic response was detected after controlling for all other variables.) As a result, deceptive orchids are likely to follow climate change more easily than nectar-rewarding species. Nevertheless, more investigations are needed to clarify the role of pollination modes in explaining phenological shifts.

Life span was found to be another important predictor of climatic responsiveness. Contrary to our expectations, long-lived species had more advanced flowering dates than short-lived species. Although this result contradicts the prediction that shorter life span might strengthen selection for the advancement of flowering (Fitter & Fitter 2002; Jump & Peñuelas 2005), it was obtained consistently from our various analyses, suggesting that phenotypic plasticity in long-lived orchids is a stronger driver of climatic responsiveness than evolutionary adaptation. Together with a similar finding by Gienapp, Leimu & Merilä (2007), this observation indicates the important role of phenotypic plasticity in shaping climatic responses over relatively short time frames. In a way, this is not surprising, as long-lived organisms are predicted to meet more diverse environmental conditions during their life than short-lived ones. Therefore, they may be better prepared to flexibly respond to environmental changes. Note, however, that presently, we possess data on the life span of orchids but

none on their relative levels of phenotypic plasticity. Researchers have just started to characterize epigenetic variation in European orchids (Paun *et al.* 2010, 2011). Since epigenetic change may underpin phenotypic plasticity, it may be crucially important in adaptation to changing environments.

Throughout various analyses, we found that the strength of phylogenetic signal did not differ markedly from zero for both measures of the advancement of flowering dates. This result parallels the results of a previous investigation showing that the advancement of spring migration among a large selection of bird species did not reflect phylogenetic signal (Végvári *et al.* 2010). The fact that we did not find phylogenetic signals in our response variables implies that sensitivity to climatic effects in Hungarian orchids can be treated as a species-specific response, free of phylogenetic inertia. Although certain life-history traits (flowering time, pollination mode, life span; see Table 1) did show a significant phylogenetic signal individually, they were not correlated and hence can be viewed as being independent of each other. Therefore, the apparently strong and opposing effects of life-history variables in closely related taxa can lower the similarity of related species and hence decrease the strength of phylogenetic signal in phenological response. The lack of overall phylogenetic inertia contrasts with the conclusions of Willis *et al.* (2008) and Davis *et al.* (2010), who studied the climate change-driven species loss within given territories. The reason for this discrepancy may lie in the contrasting taxonomic coverage of the studies: the above-mentioned works included a wide range of plant species sampled from several taxonomic families, whereas the species included in this study are less taxonomically diverse. Taking this question one step further, we can ask, might phylogenetic signals only influence flowering phenology at higher taxonomic levels (i.e. among families)? This question clearly warrants further investigation.

Our analyses seem to suggest that flowering time is not a robust predictor of climatic response in orchids, as it proved to be important only in shaping the temporal trend in flowering date of the studied orchids, but it had no strong effect on the shift in flowering date after 1960 (Table 3). This is especially interesting because in other studies (e.g. Sparks, Jeffree & Jeffree 2000; Cleland *et al.* 2007), flowering time was found to be an important factor influencing phenological response. However, we feel that this discrepancy might be caused by the relatively small number of taxa in our study. Clearly, more research is needed to verify the importance of flowering time in predicting phenological response in orchids.

Biogeography was found to be moderately related to the advancement of flower production in orchids; species with a Mediterranean distribution tended to accelerate flowering more than non-Mediterranean species. The reason for this difference is currently unclear, but a Mediterranean type of distribution might indicate a life-history adaptation that benefits from the recent climate change through, for example, photoperiod, temperature cues, vernalization or precipitation (Forrest & Miller-Rushing 2010).

Our analyses revealed no influence of altitudinal distribution on the advancement of flowering, implying that this life-

history component has a minor impact on response to climate change in the orchids investigated here. This is interesting because one would expect species at higher altitudes to show stronger phenological advancements through time because temperatures are increasing at a faster rate at higher altitudes (Lenoir *et al.* 2008). Nevertheless, we do not claim that altitude may have no importance in general, given that our records represent a geographical region where the altitudinal range is relatively small (76–1014 m a.s.l.).

Although our study may have been adversely affected by varying sampling frequency, the two measures of historical phenological responses used here yielded consistent results. As the cumulative temperature between January and May showed a marked increase in Hungary during the study period, our findings indicate that temperature may (e.g. through vernalization) play an important, but certainly not exclusive, role in the advancement of flowering time in orchids (cf. Miller-Rushing & Primack 2008; Primack & Miller-Rushing 2011; Robbirt *et al.* 2011).

The differences in the relative importance of predicting factors using the two measures of phenological change could stem from the fact that these two measures might be affected differently by variation in sampling intensity through time. Although records for most species originate from relatively long periods (Table S2 in Supporting Information), there were year-to-year variations in sampling intensity and some gaps in the recording activity. Considering these potential problems, we believe that comparing flowering times between the first and second parts of the sampling period is less affected by sampling bias and hence provides a more reliable estimate of the advancement of flowering date. In addition, our measure of temporal trend is based on the ranks of flowering time, and hence, it eliminates some of the variation. In contrast, the shift in mean flowering date uses the actual number of days of change and might be a more sensitive measure of phenological response.

In summary, we have demonstrated in a diverse set of orchid species that changes in flowering phenology through time (which is associated with increased temperatures in the study area) seem to be affected most strongly by pollination mode and life span. According to our findings, deceptive or autogamous, long-lived, or early flowering, terrestrial orchids with mainly Mediterranean distributions (in our data set this category is exemplified by *O. simia* or *A. pyramidalis*) follow the changing climate more closely, at least in Hungary. Meanwhile, their later flowering, nectar-rewarding or short-lived counterparts with non-Mediterranean distribution type (as exemplified by *Dactylorhiza viridis*) do not or less markedly respond to these changes.

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References

Ahas, R., Aasa, A., Menzel, A., Fedotova, V.G. & Scheifinger, H. (2002) Changes in European spring phenology. *International Journal of Climatology*, **22**, 1727–1738.

Anderson, S.H., Kelly, D., Ladley, J.J., Molloy, S. & Terry, J. (2011) Cascading effects of bird functional extinction reduce pollination and plant density. *Science*, **331**, 1068–1071.

Araújo, M.B. & Luoto, M. (2007) The importance of biotic interactions for modelling species distributions under climate change. *Global Ecology and Biogeography*, **16**, 743–753.

Askeyev, O.V., Sparks, T.H., Askeyev, I.V., Tishin, D.V. & Tryjanowski, P. (2010) East versus West: contrasts in phenological patterns? *Global Ecology and Biogeography*, **19**, 783–793.

Baker, H.G. (1974) The evolution of weeds. *Annual Review of Ecology and Systematics*, **5**, 1–24.

Baldwin, B.G., Sanderson, M.J., Porter, J.M., Wojciechowski, M.F., Campbell, C.S. & Donoghue, M.J. (1995) The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Garden*, **82**, 247–277.

Bartomeus, I., Ascher, J.A., Wagner, D., Danfortd, B.N., Colla, S., Kornbluth, S. & Winfree, R. (2011) Climate-associated phenological advances in bee pollinators and bee-pollinated plants. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 20645–20649.

Bateman, R.M., Smith, R.J. & Fay, M.F. (2008) Morphometric and population genetic analyses elucidate the origin, evolutionary significance and conservation implications of *Orchis × angusticruris* (*O. purpurea × O. simia*), a hybrid orchid new to Britain. *Botanical Journal of the Linnean Society*, **157**, 687–711.

Bateman, R.M., Hollingsworth, P.M., Preston, J., Lou, Y.B., Pridgeon, A.M. & Chase, M.W. (2003) Molecular phylogenetics and evolution of Orchidinae and selected Habenariinae (Orchidaceae). *Botanical Journal of the Linnean Society*, **142**, 1–40.

Bateman, R.M., Hollingsworth, P.M., Squirrell, J. & Hollingsworth, M.L. (2005) Tribe Neottieae. Phylogenetics. *Genera Orchidacearum, Volume 4. Epidendroideae (part one)* (eds A.M. Pridgeon, P.J. Cribb, M.W. Chase & F.N. Rasmussen), pp. 487–495. Oxford University Press, Oxford.

Bonan, G.B. (2008) Forests and climate change: forcings, feedbacks, and the climate benefits of forests. *Science*, **320**, 1444–1449.

Burnham, K.P. & Anderson, D.R. (2002) *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. Springer Verlag, New York, NY.

Claessens, J. & Kleynen, J. (2011) *The Flower of the European Orchid. Form and Function*. Jean Claessens & Jacques Kleynen, Geuelle.

Cleland, E.E., Chuine, I., Menzel, A., Mooney, H.A. & Schwartz, M.D. (2007) Shifting plant phenology in response to global change. *Trend in Ecology & Evolution*, **22**, 357–365.

Dafni, A. (1984) Mimicry and deception in pollination. *Annual Review of Ecology, Evolution, and Systematics*, **15**, 259–278.

Davis, C.C., Willis, C.G., Primack, R.B. & Miller-Rushing, A.J. (2010) The importance of phylogeny to the study of phenological response to global climate change. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **365**, 3201–3213.

Dixon, K.W. (2009) Pollination and restoration. *Science*, **325**, 571–573.

Dunne, J.A., Harte, J. & Taylor, K.J. (2003) Subalpine meadow flowering phenology responses to climate change: Integrating experimental and gradient methods. *Ecological Monographs*, **73**, 69–86.

Fitter, A.H. & Fitter, R.S.R. (2002) Rapid changes in flowering time in British plants. *Science*, **296**, 1689–1691.

Forrest, J. & Miller-Rushing, A.J. (2010) Toward a synthetic understanding of the role of phenology in ecology and evolution. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **365**, 3101–3112.

Freckleton, R.P., Harvey, P.H. & Pagel, M. (2002) Phylogenetic analysis and comparative data: a test and review of evidence. *American Naturalist*, **160**, 712–726.

Gienapp, P., Leimu, R. & Merilä, J. (2007) Responses to climate change in avian migration time — microevolution versus phenotypic plasticity. *Climate Research*, **35**, 25–35.

Gulyás, G., Sramkó, G., Molnár V., A., Rudnóy, S., Illyés, Z., Balázs, T. & Bratek, Z. (2005) Nuclear ribosomal DNA ITS paralogs as evidence of recent interspecific hybridization in the genus *Ophrys* (Orchidaceae). *Acta Biologica Cracoviensia Series Botanica*, **47**, 61–67.

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**, 95–98.

Harmon, L., Weir, J., Brock, C., Glor, R., Challenger, W. & Hunt, G. (2009). geiger: Analysis of evolutionary diversification. R package version 1.3-1. http://CRAN.R-project.org/package=geiger

Hegland, S.J., Nielsen, A., Lázaro, A., Bjerknes, A.L. & Totland, Ø. (2009) How does climate warming affect plant–pollinator interactions? *Ecology Letters*, **12**, 184–195.

Hoffmann, A.A. & Sgrò, C.M. (2011) Climate change and evolutionary adaptation. *Nature*, **470**, 479–485.

Horváth, F., Dobolyi, Z.K., Morschhauser, T., Lőkös, L., Karas, L. & Szerdahelyi, T. (1995) *Flóra Database 1.2*. Ecological and Botanical Institute of the Hungarian Academy of Sciences, Vácrátót, Hungary.

Internicola, A.I., Bernasconi, G. & Gigord, L.D. (2008) Should food-deceptive species flower before or after rewarding species? An experimental test of pollinator visitation behaviour under contrasting phenologies. *Journal of Evolutionary Biology*, **21**, 1358–1365.

Internicola, A.I. & Harder, L.D. (2012) Bumble-bee learning selects for both early and long flowering in food-deceptive plants. *Proceedings of the Royal Society of London. Series B*, **279**, 1538–1543.

IPCC. (2007) *Intergovernmental Panel on Climate Change: Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, UK.

Jacquemyn, H., Brys, R., Honnay, O. & Hutchings, M.J. (2002) *Orchis mascula* (L.) L. Biological Flora of the British Isles No. 252. *Journal of Ecology*, **97**, 360–377.

Jacquemyn, H., Brys, R., Hermy, M. & Willems, J.H. (2005) Does nectar reward affect rarity and extinction probabilities of orchid species? An assessment using historical records from Belgium and the Netherlands. *Biological Conservation*, **121**, 257–263.

Jersáková, J., Johnson, S.D. & Kindlmann, P. (2006) Mechanisms and evolution of deceptive pollination in orchids. *Biological Reviews*, **81**, 219–235.

Jump, A.S. & Peñuelas, J. (2005) Running to stand still: adaptation and the response of plants to rapid climate change. *Ecology Letters*, **8**, 1010–1020.

Kull, T. & Hutchings, M.J. (2006) A comparative analysis of decline in the distribution ranges of orchid species in Estonia and the United Kingdom. *Biological Conservation*, **129**, 31–39.

Lenoir, J., Gegout, J.C., Marquet, P.A., de Ruffray, P. & Brisse, H. (2008) A significant upward shift in plant species optimum elevation during the 20th century. *Science*, **320**, 1768–1771.

Martins, E.P. & Hansen, T.F. (1997) Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. *American Naturalist*, **149**, 646–667.

Menzel, A., Sparks, T.H., Estrella, N. & Roy, D.B. (2006) Altered geographic and temporal variability in phenology in response to climate change. *Global Ecology and Biogeography*, **15**, 498–504.

Miller-Rushing, A.J., Inouye, D.W. & Primack, R.B. (2008) How well do first flowering dates measure plant responses to climate change? The effects of population size and sampling frequency. *Journal of Ecology*, **96**, 1289–1296.

Miller-Rushing, A.J. & Primack, R.B. (2008) Global warming and flowering times in Thoreau's Concord: a community perspective. *Ecology*, **89**, 332–341.

Miller-Rushing, A.J., Primack, R.B., Primack, D. & Mukunda, S. (2006) Photographs and herbarium specimens as tools to document phenological changes in response to global warming. *American Journal of Botany*, **93**, 1667–1674.

Molnár V., A. (ed.) (2011) *Magyarország orchideáinak atlasza [Atlas of Hungarian Orchids]*. Kossuth Kiadó, Budapest.

Molnár V., A., Takács, A., Horváth, O., Vojtkó, A.E., Király, G., Sonkoly, J. & Sramkó, G. (2012) Herbarium Database of Hungarian Orchids I. Methodology, dataset, historical aspects and taxa. *Biologia*, **67**, 79–86.

Neiland, M.R.M. & Wilcock, C.C. (1999) Fruit set, nectar reward, and rarity in the Orchidaceae. *American Journal of Botany*, **85**, 1657–1671.

Orme, D., Freckleton, R., Thomas, G., Petzoldt, T. & Fritz, F. (2009) *CAIC: Comparative Analyses using Independent Contrasts*. http://R-Forge.R-project.org/projects/caic/

Pagel, M. (1997) Inferring evolutionary processes from phylogenies. *Zoologica Scripta*, **26**, 331–348.

Pagel, M. (1999) The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. *Systematic Biology*, **48**, 612–622.

Panchen, Z.A., Primack, R.B., Anisko, T. & Lyons, R.E. (2012) Herbarium specimens, photographs, and field observations show Philadelphia area plants are responding to climate change. *American Journal of Botany*, **99**, 751–756.

Parmesan, C. (2006) Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, **37**, 637–669.

Parmesan, C. & Yohe, G. (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, **421**, 37–42.

Paun, O., Bateman, R.M., Fay, M.F., Hedrén, M., Civeyer, L. & Chase, M.W. (2010) Stable epigenetic effects impact adaptation in allopolyploid orchids (*Dactylorhiza*: Orchidaceae). *Molecular Biology and Evolution*, **27**, 2465–2473.

Paun, O., Bateman, R.M., Fay, M.F., Luna, J.A., Moat, J., Hedrén, M. & Chase, M.W. (2011) Altered gene expression and ecological divergence in sibling allopolyploids of *Dactylorhiza* (Orchidaceae). *BMC Evolutionary Biology*, **11**, 113.

Pellissier, L., Vittoz, P., Internicola, A.I. & Gigord, L.D.B. (2010) Generalized food-deceptive orchid species flower earlier and occur at lower altitudes than rewarding ones. *Journal of Plant Ecology*, **3**, 243–250.

Post, E.S., Pedersen, C., Wilmers, C.C. & Forchhammer, M.C. (2008) Phenological sequences reveal aggregate life-history response to climatic warming. *Ecology*, **89**, 363–370.

Prather, L.A., Alvarez-Fuentes, O., Mayfield, M.H. & Fergusson, C.J. (2004) The decline of plant collecting in the United States: a threat to the infrastructure of biodiversity studies. *Systematic Botany*, **29**, 15–28.

Price, M.W. & Waser, N.M. (1998) Effects of experimental warming on plant reproductive phenology in a subalpine meadow. *Ecology*, **79**, 1261–1271.

Primack, R.B. & Miller-Rushing, A.J. (2009) The role of botanical gardens in climate change research. *New Phytologist*, **182**, 303–313.

Primack, R.B. & Miller-Rushing, A.J. (2011) Broadening the study of phenology and climate change. *New Phytologist*, **191**, 307–309.

R Development Core Team. (2010) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Rafferty, N.E. & Ives, A.R. (2011) Effects of experimental shifts in flowering phenology on plant-pollinator interactions. *Ecology Letters*, **14**, 69–74.

Robbirt, K.M., Davy, A.J., Hutchings, M.J. & Roberts, D.L. (2011) Validation of biological collections as a source of phenological data for use in climate change studies: a case study with the orchid *Ophrys sphegodes*. *Journal of Ecology*, **99**, 235–241.

Root, T.L., Price, J.T., Hall, K.R., Schneider, S.H., Rosenzweig, C. & Pounds, J.A. (2003) Fingerprints of global warming on wild animals and plants. *Nature*, **421**, 57–60.

Sanderson, M.J. (2003) r8s: inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. *Bioinformatics*, **19**, 301–302.

Schiestl, F.P. (2005) On the success of a swindle: pollination by deception in orchids. *Naturwissenschaften*, **92**, 255–264.

Schröter, D., Cramer, W., Leemans, R., Prentice, I.C., Araújo, M.B., Arnell, N. W. *et al.* (2005) Ecosystem service supply and vulnerability to global change in Europe. *Science*, **310**, 1333–1337.

Schwartz, M.D., Ahas, R. & Aasa, A. (2006) Onset of spring starting earlier across the Northern hemisphere. *Global Change Biology*, **12**, 343–351.

Sherry, R.A., Zhou, X.H., Gu, S.L., Arnone, J.A., Schimel, D.S., Verburg, P. S., Wallace, L.L. & Luo, Y. (2007) Divergence of reproductive phenology under climate warming. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 198–202.

Sparks, T.H., Jeffree, E.P. & Jeffree, C.E. (2000) An examination of the relationship between flowering times and temperature at the national scale using long-term phenological records from the UK. *International Journal of Biometeorology*, **44**, 82–87.

Sparks, T.H. & Menzel, A. (2002) Observed changes in seasons: an overview. *International Journal of Climatology*, **22**, 1715–1725.

Swarts, N.D. & Dixon, K.W. (2009) Terrestrial orchid conservation in the age of extinction. *Annals of Botany*, **104**, 543–556.

Swofford, D.L. (2002) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4*. Sinauer Associates, Sunderland, MA.

Vallius, E. (2000) Position-dependent reproductive success of flowers in *Dactylorhiza maculata*. (Orchidaceae). *Functional Ecology*, **14**, 573–579.

Végvári, Z., Bókony, V., Barta, Z. & Kovács, G. (2010) Life-history predicts advancement of avian spring migration in response to climate change. *Global Change Biology*, **16**, 1–11.

Walther, G.R. (2004) Plants in a warmer world. *Perspectives in Plant Ecology, Evolution, and Systematics*, **6**, 169–185.

Walther, G.R. (2010) Community and ecosystem responses to recent climate change. *Philosophical Transactions of the Royal Society of London Series B*, **365**, 2019–2024.

Walther, G.-R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., Fromentin, J.-M., Hoegh-Guldberg, O. & Bairlein, F. (2002) Ecological responses to recent climate change. *Nature*, **416**, 389–395.

Willis, C.G., Ruhfel, B., Primack, R.B., Miller-Rushing, A.J. & Davis, C.C. (2008) Phylogenetic patterns of species loss in Thoreau's woods are driven by climate change. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 17029–17033.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. List of taxa and source of nrITS sequences used in this study.

Table S2. Data used in the analyses.

Table S3. Results of alternative threshold values for calculating shift in flowering phenology.

Table S4. Bivariate relationships among pairs of life-history variables.

Table S5. Results with *Spiranthes spiralis* included.

Table S6. Results controlling for elevation and latitude.

Figure S1. Maximum parsimony phylogenetic tree used in this study.

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