

REVIEW AND SYNTHESIS

Meta-analysis of phenotypic selection on flowering phenology suggests that early flowering plants are favoured

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Abstract

Flowering times of plants are important life-history components and it has previously been hypothesized that flowering phenologies may be currently subject to natural selection or be selectively neutral. In this study we reviewed the evidence for phenotypic selection acting on flowering phenology using ordinary and phylogenetic meta-analysis. Phenotypic selection exists when a phenotypic trait co-varies with fitness; therefore, we looked for studies reporting an association between two components of flowering phenology (flowering time or flowering synchrony) with fitness. Data sets comprising 87 and 18 plant species were then used to assess the incidence and strength of phenotypic selection on flowering time and flowering synchrony, respectively. The influence of dependence on pollinators, the duration of the reproductive event, latitude and plant longevity as moderators of selection were also explored. Our results suggest that selection favours early flowering plants, but the strength of selection is influenced by latitude, with selection being stronger in temperate environments. However, there is no consistent pattern of selection on flowering synchrony. Our study demonstrates that phenotypic selection on flowering time is consistent and relatively strong, in contrast to previous hypotheses of selective neutrality, and has implications for the evolution of temperate floras under global climate change.

Keywords

Flowering phenology, flowering synchrony, flowering time, mutualisms, natural selection, phenotypic selection.

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INTRODUCTION

Natural selection is thought to be a major force driving adaptation and evolutionary change in nature (Endler 1986). However, recent multi-taxa reviews of standardized estimations of phenotypic selection (i.e. selection gradients) have shown that selection in natural populations is frequently weak, with just a few cases reporting strong selection (Hoekstra *et al.* 2001; Kingsolver *et al.* 2001; Kingsolver & Pfennig 2007). In many plants and other organisms timing of reproduction has great impact on their reproductive success (e.g. Landa 1992; Thomas *et al.* 2001; Dickerson *et al.* 2005; Forrest & Thomson 2010). Despite the evidence of recurrently weak selection in nature, the hypothesis that flowering phenologies have been shaped by natural selection has long appealed to ecologists (Kudo 2006; Elzinga *et al.* 2007). Plant populations show interindividual variation in their reproductive schedules (e.g. Ehrlén & Münzbergová 2009) and this variation has a genetic basis in most plant species (McMillan & Pagel 1958; McIntyre & Best 1978; Mazer & LeBuhn 1999; Van Dijk & Hautekeete 2007; Wilczek *et al.* 2009). Genetically-based natural variation is the raw material for contemporary natural selection; this selection is detected when a trait co-varies with fitness and it has been identified in several plant populations (e.g. Sandring *et al.* 2007;

Sandring & Ågren 2009; Forrest & Thomson 2010). However, whether phenology is currently subject to natural selection or is selectively neutral has been the subject of some debate (e.g. Ollerton & Lack 1992, 1993; Fox & Kelly 1993). Net selection or the response to selection on flowering phenology can be relaxed or nullified (*sensu* Ollerton & Lack 1992) by the action of: (1) multiple competing factors (e.g. herbivores, pollinators, resources; Gómez 1993); (2) phylogenetically influenced traits or constraints (Kochmer & Handel 1986; Ollerton & Lack 1992; Johnson 1993; Smith-Ramírez *et al.* 1998; Ollerton & Diaz 1999) such as number of reproductive events (annual, perennial), seasonal timing of flowering (spring, autumn), and others that may affect the strength of, or the response to, selection; (3) or because changes in phenology must be compatible with other time-dependent processes such as growth, seed development and seed dispersal (Primack 1987).

Current opinion about whether flowering phenology is generally subject to phenotypic selection or is selectively neutral is contentious and therefore a rigorous review on the topic with suitable methods is needed. To our knowledge this topic has been the focus of only a few narrative reviews (Kudo 2006; Elzinga *et al.* 2007) and one quantitative (vote counting) review that looked at the evidence for selection and adaptation on a set of floral traits including unspecified

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components of phenology (Harder & Johnson 2009). The latter review found that for flowering phenology, selection gradients were not significant in more than 60% of the studies which apparently suggests that, as in other traits, phenotypic selection on flowering phenology is weak or negligible in most cases (Hoekstra *et al.* 2001; Kingsolver *et al.* 2001). Flowering phenology is a multivariate trait that can be measured in different ways (e.g. flowering onset, duration, end of flowering), not all of which may be subject to the same selection. Therefore, in our study we looked at phenotypic selection on different components of flowering phenology separately (see below). We also assessed the effects of sample size, because Harder & Johnson (2009) identified a relationship between sample size and significance of selection gradients, and publication bias (identified in studies reviewed by Kingsolver *et al.* 2001; Kingsolver & Pfennig 2007), both of which may influence the results obtained from a quantitative review. Previous reviews did not take into account the shared histories of species which has been shown to affect the flowering schedule of some plants (Kochmer & Handel 1986; Johnson 1993; Smith-Ramírez *et al.* 1998). Analysing data from different species (Felsenstein 1985) and studies (Hedges & Olkin 1985) with standard statistical methods can produce misleading results. Fortunately, recently developed phylogenetic meta-analysis approaches cope with all of the above issues (Adams 2008; Lajeunesse 2009). This is a promising approach which will help ecologists and evolutionary biologists improve our understanding of patterns of phenotypic selection.

In this study we assessed the patterns of phenotypic selection on flowering phenology with both ordinary and phylogenetic meta-analysis, in relation to ecological and life-history traits which previous work has suggested will affect the incidence and strength of phenotypic selection among taxa. We focused our analysis on two specific phenological traits that selection may target: flowering time and flowering synchrony, which are the most studied descriptors of phenology. Although synchrony is not a proper individual characteristic, it can be seen as an example of multilevel selection, in which the variation in the fitness of individuals is due to both properties of the individuals as well as the properties of the population to which they belong (Stevens *et al.* 1995). Therefore, flowering synchrony can arise, persist or be selected against via natural selection based on individual reproductive success increasing or decreasing as a function of simultaneously flowering individuals (e.g. Parra-Tabla & Vargas 2007). It is known that several factors may affect the ecology and evolution of flowering phenologies, including features of the mating environment during flowering, herbivory on flowers and developing seeds, length of the growing and reproductive season, abiotic conditions that affect mating and seed production, as well as storage of resources needed for reproduction (reviewed by Kudo 2006). Therefore, we looked at the effect of a suite of life-history traits (plant size, number and duration of reproductive episodes), species interactions (dependence on pollinators, type of pollen vector and pre-dispersal seed predation) and environmental factors (latitude) on the incidence and strength of phenotypic selection on flowering time and flowering synchrony. Previous studies have looked at the role of several of these variables as moderators of selection on flowering time and/or flowering synchrony of only a limited range of species, with varying results, such that it is impossible to make a global prediction. Therefore, for a better understanding we are adopting an exploratory approach that we will compare to previous results in the Discussion.

MATERIALS AND METHODS

The search

A review of the available literature was carried out using 'phenotypic selection' and 'flowering phenology' as key words in the following online databases: Google Scholar (<http://www.scholar.google.com>), ISI Web of Knowledge (<http://www.isiwebofknowledge.com>), JStor (<http://www.jstor.org>), SciELO (<http://www.scielo.org>) and Scopus (<http://www.scopus.com>). The search was not limited by year or journal. Once the papers were obtained, the references therein were examined in order to find earlier publications. We also asked colleagues working on the topic for unpublished data and used our own unpublished studies in the analysis.

Study selection criteria and phenotypic selection on phenology

We selected those studies where the relationship between flowering onset and/or flowering synchrony and fitness was shown. Flowering time was usually reported as either the calendar date or the relative time (e.g. mean date of flowering or flowering rank) an individual starts producing flowers. Flowering synchrony among individuals was reported in virtually all studies we reviewed as Augspurger's (1983) index; only two studies used different indexes but with the same scale as that of Augspurger's index. In most of the studies we reviewed, female fitness was reported as fruit or seed production. Pollen deposition (two cases) and finite growth rate (one case) were rarely reported. Male fitness was reported in some studies (five cases) along with female fitness; in these cases, male and female fitness were averaged or—when it was available—a composite fitness estimation (i.e. a fitness measure where both male and female fitness are considered) was preferred. Although lifetime fitness was evaluated in just eight species, fitness was evaluated in at least two reproductive episodes for nearly half (43%) of species considered in the analysis. Most studies included in our analyses were non-manipulative; for manipulative studies, data from control groups was used for effect size computation. We did not include any study which artificially manipulated flowering time phenotypes. Variation in methodological issues (e.g. study type, type of fitness or synchrony index used) were not addressed in our study because these were largely homogeneous in our data set and because our study is focused only on ecologically relevant moderator variables. Traditionally, selection on a given trait has been described by regression (Lande & Arnold 1983) or correlation (e.g. Primack 1980; Ollerton & Lack 1998) coefficients of the relationship between a character and fitness. Therefore, we considered Pearson's r as the most straightforward metric of the effect size to assess selection on flowering time and flowering synchrony. When selection gradients or selection differentials (*sensu* Lande & Arnold 1983) were reported, these were transformed into r as long as any measure of dispersion and sample size, or the phenotypic variance-covariance matrix, were available. Values for Pearson's r were also obtained from a variety of summarizing statistics (F , Z , t , χ^2) or from one tail P -values when sample size was known (Rosenthal 1991). In the studies where nonparametric correlations (Kendall or Spearman) were used, we calculated r from P -values. In two cases (Ollerton & Lack 1998; Salinas-Peba & Parra-Tabla 2007) we went back to the raw data and r was calculated directly from that data set. When information needed was available only in published plots, we accessed them with DATA THIEF II (<http://www.datathief.org>) which is software to reverse engineer the raw data. When information from which r can

be obtained was presented in more than two forms in a study, we preferred the form which needed the simpler algorithm to calculate r . Unlike selection gradients, selection differentials, simple regression and correlation analyses do not explicitly address direct versus indirect selection; however, all the reviews on the topic agree that, in most cases, total selection and direct selection match in direction (Endler 1986; Hoekstra *et al.* 2001; Kingsolver *et al.* 2001; Harder & Johnson 2009). Therefore, all these measures of selection should give qualitatively similar information. Studies from which we did not get the full information (in any form, numeric or in figures) needed to calculate r , were excluded from the analyses.

Pearson's r was obtained or estimated from all the subsets of data provided by authors in the original study (e.g. more than one population) and each of these was transformed to Zr using Fisher's algorithm (Hedges & Olkin 1985). However, as we decided to perform the meta-analysis at species level, only a single mean effect size was calculated for each species by averaging the Zr values (Rosenthal 1991). As in previous meta-analyses at species level, in studies providing data for more than one species, each species was considered as an independent effect size (e.g. Aguilar *et al.* 2006; Munguía-Rosas *et al.* 2009; Morales & Traveset 2010). When the same species had been studied in more than one research project, Zr values were averaged. Sometimes studies provided directional as well as disruptive or stabilizing selection gradients/differentials, and in these cases the selection gradient or differential with better fit (narrower confidence interval or standard error) was selected. However, stabilizing or disruptive selection gradients only rarely (four cases) showed a better fit than directional. Stabilizing and disruptive selection are addressed statistically with quadratic regression models (Lande & Arnold 1983); therefore, we calculated r from the statistics of the quadratic term.

Once we had obtained and transformed the effect sizes per species, the overall effect size was calculated for two effects: the relationship between flowering time and any estimation of fitness (hereafter selection on flowering time) and the relationship between flowering synchrony and fitness (hereafter selection on flowering synchrony). The overall effect size was calculated with ordinary random-effects meta-analysis (hereafter ordinary meta-analysis) and phylogenetic random-effects meta-analysis (hereafter phylogenetic meta-analysis). Random-effects models calculate an average effect size from a sample of effect sizes in contrast to the assumption of only one real effect size in the case of fixed-effect models. That is, when studies are significantly heterogeneous (as usually seen in ecology) random-effects models are preferred; the classical measure of heterogeneity is Cochran's Q , which is calculated as the weighted sum of squared differences between individual study effects and the pooled effect across studies (Gurevitch & Hedges 1999). Analytically, the difference between models is essentially the estimated variance: fixed effects only consider the within-study variance while random models consider the between-study variance (τ^2) as well. To achieve a more accurate approximation to the distribution of Zr , the asymptotic within-species variance $1/(n-3)$ – where n is the sample size – was preferred (Hedges & Olkin 1985). The inverse of variance was used as a vector of weights in the analyses; as variance is inversely proportional to sample size, studies with larger sample sizes have relatively more weight in analyses than studies with smaller sample sizes.

Ordinary meta-analysis and phylogenetic meta-analysis are specialized cases of generalized theory of least squares (Lajeunesse 2009), a framework that addresses directly violations due to non-independence

and heteroscedasticity of data. These violations are explicitly modelled in a $k \times k$ covariance matrix (Σ):

$$E \sim N(X \delta, \Sigma)$$

where E is a $k \times 1$ vector of k number of effect sizes (δ), which are assumed to be normally distributed (N). X is the design matrix where moderator variables are codified; when only the overall effect size needs to be estimated, X becomes a vector of 1's. In ordinary meta-analysis Σ contains the sampling variances of each effect size on its main diagonal, modelling a weighted least squares regression where effect sizes with large variances are penalized during the pooling of effect size. In the comparative method, phylogenetic relatedness is provided in off diagonal elements of Σ . The novel approach of phylogenetic meta-analysis takes advantage of the underlying shared theory of both approaches: the meta-analysis and the comparative method (Adams 2008; Lajeunesse 2009). Adams (2008) implemented the phylogenetic meta-analysis converting the meta-analytical data (E and X) into phylogenetically independent data (E_{new} and X_{new} in Adams' notation) multiplying meta-analytical data by D – a matrix produced by singular decomposition of Σ . Then a meta-analysis is performed using a weighted regression model. The Adams' approach was implemented in R (The R Development Core Team 2007) and updated on 29 April 2009 (<http://www.public.iastate.edu/~dcadams/software.html>). We modified the Adams' R code slightly to perform random-effects models introducing as weights the inverse of τ^2 plus the within-species variance. The significance of coefficients was assessed contrasting the value of the coefficients against Z -values. An improvement of this method has been developed by Lajeunesse (2009); the updated Adams' and Lajeunesse's approaches lead to similar results, but Lajeunesse (2009) designed some algorithms to estimate among-species heterogeneity, random-effects models, different evolutionary hypotheses and model contrasts with the Akaike Information Criterion (AIC). Ordinary and phylogenetic meta-analyses are competing hypotheses (Lajeunesse 2009) and the AIC is a criterion that seeks a model that has a good fit but few parameters (Crawley 2007). Some of these algorithms were implemented in a pilot software project called PHYLOMETA (<http://lajeunesse.nescent.org/software.html>). In this study the two currently available approaches were used.

The publication bias of this research topic was assessed both graphically, by drawing funnel plots, and statistically with regression methods (Egger *et al.* 1997) implemented in the Metafor package for R (Viechtbauer 2010). Metafor was also used to estimate overall effect size of ordinary meta-analysis; this package uses weighted least squares regression as in Adams (2008) approach.

Factors affecting selection on phenology

We evaluated the influence of a number of moderator variables on the calculated effect sizes. These variables were: dependence on pollinators, longevity of plants, identity of the pollen vector, latitude and duration of flowering season at population level. The dependence on pollinators was scored using a scale from one to five, one being the strongest and five the weakest dependence on pollinators. The values were assigned as follows: dioecious plants (1); self incompatible + floral condition preventing self-pollination (e.g. distylous, protandrous etc.) (2); self incompatible and no reported floral condition preventing self-pollination (3); self-compatible + floral condition preventing self-pollination (4); self-compatible and no reported floral condition

preventing self-pollination (5). We consider longevity in terms of reproductive events, that is, perennials with more than two reproductive events in one group (perennials) and annuals, biennials and monocarpic perennials in another (short-lived). The vectors of pollen were categorized as 'animal-pollinated' and 'wind-pollinated' plants. In most of the multi-population studies, the populations were within the same degree of latitude; only for two species were the studied populations more than one degree of latitude apart [*Arabidopsis lyrata* (L.) and *Lythrum salicaria* (L.) O'Kane & Al-Shehbaz]; in these two cases, effect sizes and latitudes were averaged. Most of the studies included information on these moderator variables in the published paper; when they did not, complementary literature was consulted or we asked authors for additional information. Herbarium specimens or photographs were also considered as complementary sources of information. The compatibility system was assigned using a large database on this topic belonging to M.M. Ferrer-Ortega (mferrer@uady.mx). All the moderator variables listed above were used to explain variation in selection on flowering time. However, in the case of selection on flowering synchrony, only dependence on pollinator, latitude and duration were taken into account owing to the lack of short-lived species and poor representation (only one example) of wind-pollinated species in data set. The effects of the moderator variables were implemented by including the variables in the matrix design (X) and the significance of coefficients was assessed by its standard error or heterogeneity (Hedges & Olkin 1985). Ordinary meta-analyses and phylogenetic meta-analyses were fitted with the Metafor package and the approach of Adams (2008), respectively. We did not assess the effect of moderator variables using the Lajeunesse (2009) approach because PHYLOMET software aborted the calculation before finishing the analyses, presumably because of problems with matrix convergence.

Effects of pre-dispersal seed predation and plant size on flowering time

It has been suggested repeatedly in the literature that both interactions with antagonists (particularly seed predators) and plant size can affect flowering time (reviewed by Kudo 2006 and Elzinga *et al.* 2007). Seed predators could affect the evolution of flowering time if those predators preferentially target seeds being produced at a particular time of the year (e.g. by early, peak or late flowering plants), whilst plant size can have an influence on flowering time because larger individuals tend to flower earlier in some species at least (e.g. Ollerton & Lack 1998). Therefore, we independently assessed the effect of these variables on flowering time using ordinary and phylogenetic meta-analyses as outlined above. We searched for studies in the databases described above using 'phenotypic selection', 'plant size' and 'pre-dispersal seed predation' as keywords. All studies showing the relationship between intensity of seed predation or a surrogate of size with flowering time, where enough information (numeric or graphical) to calculate r was given, were included in our survey. Seed predation was carried out by a wide variety of predators, typically insects, and studies assessing predation on developing seeds, mature seeds and fruits were included in the analysis. Plant size was frequently measured as plant height; far less frequently, size was reported as number of leaves (three cases), stem diameter (one case), plant volume (two cases) or a composite index (one case). Owing to the homogeneity in methodological (e.g. plant size surrogate) and biological issues (e.g. life form) seen in the reviewed studies, no moderator variable was

considered in this analysis. Publication bias was once again assessed with funnel plots and using regression techniques (Egger *et al.* 1997).

Phylogenetic framework

To perform phylogenetic meta-analysis a phylogenetic hypothesis is required. An initial scheme was constructed using the online tool Phylomatic (<http://www.phylodiversity.net/phyloomatic>), based on the Angiosperm super tree built by Davis *et al.* (2004). This allows one to input a list of plant species with their family affiliation to obtain a phylogenetic tree. We selected the 'conservative seed plant tree' option, which leaves nodes with less than 80% support as soft polytomies. After that, the topology was entirely resolved with the help of several studies for Asteraceae (Jansen *et al.* 1991), Cactaceae (Taylor & Zappi 1989; Nyffeler 2002; Arias *et al.* 2003), Caryophyllaceae (Popp & Oxelman 2004; Fior *et al.* 2006), Ericaceae (Kron 1997; Kron *et al.* 2002), Fabaceae (Kajita *et al.* 2001), Orchidaceae (Cameron 2007), Poaceae (GPWG 2000; Schneider *et al.* 2009), Polemoniaceae (Johnson *et al.* 2008), and Ranunculaceae (Ro & McPherson 1997; Wang *et al.* 2009). Relationships for species in the genus *Solidago* were resolved using information from internal transcribed spacer (ITS) sequences (Genbank accessions: AF046982, DQ005981, EU125357, FJ859719, FJ980344) using an exhaustive parsimony analysis conducted in PAUP 4.0b10 (Swofford 2003). As several species have not been taken into account in any published study, the branch length is unknown. To handle this issue a branch length of one was assumed, as in previous phylogenetic meta-analyses facing this situation (Verdú & Traveset 2004, 2005; Munguía-Rosas *et al.* 2009). Initially we built a primary phylogeny considering all the species and all the effects (Fig. 1). Then species needed for specific analyses were selected by trimming unneeded branches in the primary phylogeny. From this tree we obtained a covariance matrix where the topology and the sum of branch distance from root to tips was used to penalize species relatedness.

RESULTS

Data sets and phenotypic selection on phenology

After careful scrutiny of 296 studies dealing with flowering phenology, data of 87 plant species from 40 families (71 studies) and 18 species from 13 families (10 studies) met the selection criteria to assess the evidence for phenotypic selection acting on flowering time and on flowering synchrony. In the flowering time data set the number of species per family ranged from one to eight, with the most represented being Asteraceae (eight species), Fabaceae (eight species), Caryophyllaceae (seven species) and Orchidaceae (six species). In the flowering synchrony data set, the number of species per family ranged from one to three, and the most represented family was Cactaceae (in this data set most of the families were represented by only one species). The lists of species, families (Appendix S1) and studies (Appendix S2) are shown as online supporting files.

Estimates of between-studies variation (τ^2) values were 0.32 for selection on flowering time and 0.02 for selection on flowering synchrony. Regardless of the data set (flowering time or flowering synchrony), statistical approach (Adams 2008 or Lajeunesse's 2009) or analysis (ordinary or phylogenetic meta-analysis), between-species heterogeneity was always statistically significant (Q_{86} ranges from 4522 to 24317 in the flowering time data set, Q_{17} ranges from 57 to 122 in the flowering synchrony data set). This justifies the selection of

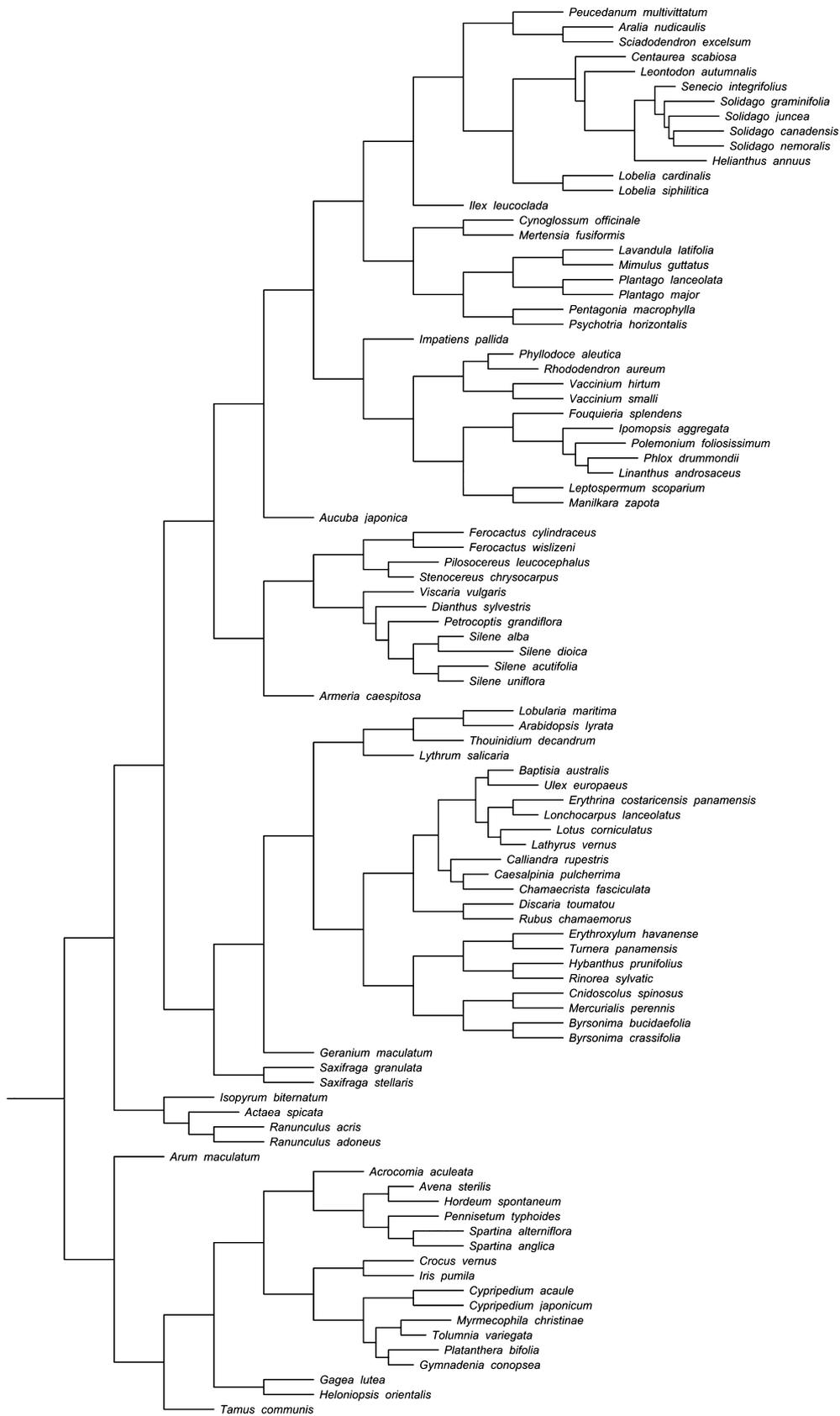


Figure 1 Phylogenetic tree of species considered in the study. All the species included in all the analyses are shown.

random-effects models. Tests of asymmetry of funnel plots accepted the null hypotheses of symmetry in the effect sizes in both data sets: flowering time ($t_{85} = -0.050$, $P = 0.960$) and flowering synchrony ($t_{16} = 1.306$, $P = 0.201$). Therefore, there is no evidence of publication bias. Funnel plots are available as online supporting information (Figure S1).

The results of the ordinary meta-analysis showed significant, negative mean selection on flowering time but no selection on flowering synchrony. Forest plots of these two effects are available in online supporting material (Figure S2) whilst effect sizes (Z_i) are shown in Appendix S2. When phylogenetic relationships were taken into account both effects – selection on flowering time and selection on flowering synchrony – were not statistically different from zero and both Adams (2008) and Lajeunesse's (2009) approaches led to consistent results. However, ordinary meta-analysis obtained the lowest AIC (better fit) in the case of selection on flowering time following the two approaches. The AIC was contradictory in the case of flowering synchrony (Table 1).

Factors affecting selection on phenology

After fitting the model with moderator variables the heterogeneity in the effect of selection on flowering time was still significant ($Q_{77} = 2290.71$, $P < 0.01$), although substantially smaller ($\tau^2 = 0.2574$) than in models without moderators. Of the moderator variables taken into account, dependence on pollen vector, plant longevity and latitude significantly explained variation in selection on flowering time in ordinary meta-analysis (Table 2). From these, dependence on pollen vector and latitude were also significant in the phylogenetic meta-analysis. Additionally, phylogenetic meta-analysis suggests that duration of flowering season significantly affects selection on flowering time (Table 2). Only the category with the lowest dependence on pollinators was statistically different from zero (estimate = -0.543 ± 0.230 , $Z = -2.38$, $P = 0.017$; Fig. 2a), which suggests stronger selection on flowering onset in those plants that do not depend on pollinators (i.e. highly selfing species). However, this category contained most of the short-lived plants; these plants exhibited a negative effect size (-0.59 ± 0.190) while perennials did

Table 1 Overall selection on two phenological descriptors (effect sizes): flowering time (onset) and flowering synchrony (synchrony). Ordinary meta-analysis (ordinary) and phylogenetic meta-analysis (phylogenetic) were carried out. The two currently available approaches to address a phylogenetic meta-analysis (Adams 2008 and Lajeunesse 2009) were implemented. Standard error (SE) of the overall estimate and associated statistics are shown. The Akaike Information Criterion (AIC) of each model is given to assess model fit, with lower values indicating a better fit

Approach	Effect	Analysis	Estimate \pm 1 SE	Statistics (Z)	AIC
Adams	Onset	Ordinary	-0.140 ± 0.065	-2.147^*	113.45
		Phylogenetic	-0.018 ± 0.418	-0.042 ns	165.74
	Synchrony	Ordinary	0.003 ± 0.049	0.070 ns	0.51
		Phylogenetic	-0.049 ± 0.142	-0.346 ns	-7.77
Lajeunesse	Onset	Ordinary	-0.139 ± 0.057	-2.420^*	271.93
		Phylogenetic	-0.134 ± 0.074	-1.807 ns	407.65
	Synchrony	Ordinary	0.004 ± 0.051	0.078 ns	51.27
		Phylogenetic	-0.028 ± 0.075	-0.374 ns	66.63

* $P < 0.05$.

ns, non-significant result.

Table 2 Results of statistical analyses to assess the influence of some moderator variables on two effect sizes: selection on flowering time (onset) and selection on flowering synchrony (synchrony). Ordinary random-effects meta-analysis (ordinary) and phylogenetic random-effects meta-analysis (phylogenetic) were carried out. The moderator variables are the degree of pollinator dependence (one to five scale, with one being the category with strongest dependence, in table 'dependence'), longevity (two levels, short-lived and perennial plants), the pollen vector (two levels, animal- and wind-pollinated plants, in table 'vector'), the latitude (a covariable) and duration of the flowering period at the population level (a covariable, in the table 'duration'). Z statistics are shown and heterogeneity among groups (Q_b) in the case of 'dependence' for ordinary meta-analysis. The AIC is also shown to assess model fit. The approach to fit and test for significance in phylogenetic meta-analysis was as outlined by Adams (2008)

Effect	Moderators	Analysis			
		Ordinary		Phylogenetic	
		Statistics	AIC	Statistics	AIC
Onset	Dependence	$Q_b = 11.95^*$	153.947	$Z = -2.653^{**}$	100.789
	Longevity	$Z = -2.666^{**}$		$Z = -1.526$ ns	
	Vector	$Z = 0.566$ ns		$Z = 0.037$ ns	
	Latitude	$Z = -2.988^{**}$		$Z = -2.243^*$	
	Duration	$Z = 1.171$ ns		$Z = 2.752^{**}$	
Synchrony	Dependence	$Q_b = 1.604$ ns	5.676	$Z = 0.216$ ns	-1.954
	Latitude	$Z = 0.228$ ns		$Z = 0.105$ ns	
	Duration	$Z = 0.703$ ns		$Z = 0.146$ ns	

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

ns, non-significant result.

not differ from zero (-0.085 ± 0.246 ; Fig. 2b). Disentangling these two factors (short life span vs. low dependence on pollinators) will require a larger data set. The relationship between latitude and effect size was negative (slope = -0.016 ± 0.005); in other words, selection on flowering time tends to be stronger in temperate plants than in tropical plants (Fig. 3). The relationship between flowering duration and selection on flowering time corrected by phylogeny showed a negative coefficient (-0.002 ± 0.001 , intercept = 0.041 ± 0.052), indicating stronger selection in plants with shorter flowering periods. No moderator variable explained variation in selection on flowering synchrony (Table 2). The AIC was consistently lower in phylogenetic meta-analyses than in ordinary analyses.

Effect of plants size and pre-dispersal seed predation on flowering time

Considering all the ordinary and phylogenetic meta-analyses as well as the two approaches (Adams' and Lajeunesse's), the resulting heterogeneity was significant for the effect of plant size on flowering time ($Q_{25} = 1903-10\ 903$, $P < 0.01$) and pre-dispersal seed predation on flowering time ($Q_{13} = 101-190$, $P < 0.01$). τ^2 value for the effect of size on flowering time was 0.565 and 0.065 for the effect of pre-dispersal seed predation on flowering time. The test of asymmetry accepted the null hypothesis of symmetry in funnel plots in both effects, plant size on flowering time ($t_{24} = 0.568$, $P = 0.574$) and pre-dispersal seed predation on flowering time ($t_{12} = -0.495$, $P = 0.629$), indicating no publication biases. Funnel and forest plot for both effects are included as online supporting material (Figures S1 and S2, respectively).

According to the results of ordinary meta-analysis, plant size has a significantly negative effect on flowering time following Lajeunesse's approach; however, this is only marginally significant ($P = 0.089$)

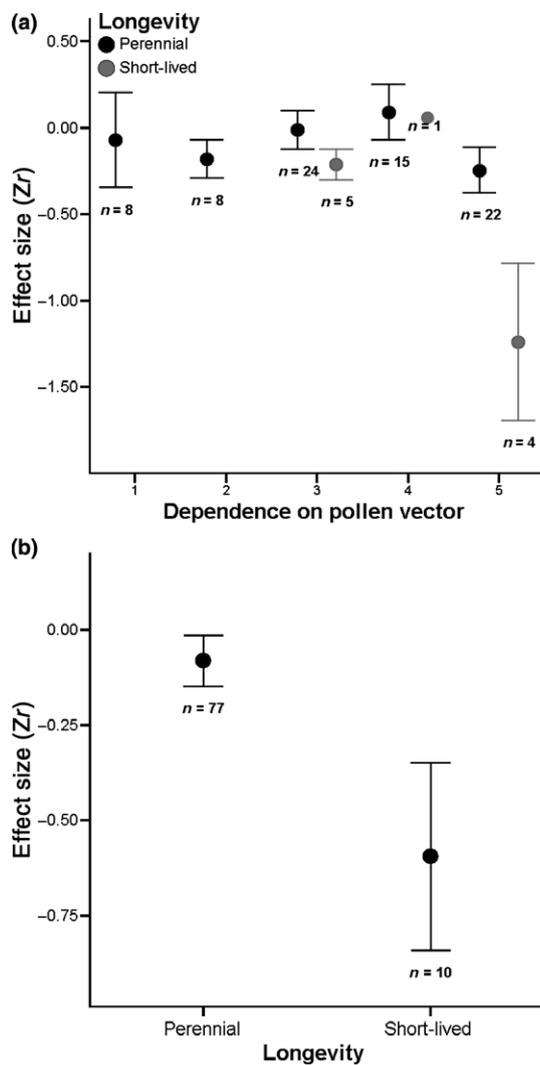


Figure 2 Selection on flowering time (effect size) of plant species with different degrees of dependence on pollinators (a) and with different longevity (b). We established five levels of pollinator dependence, 1 being the strongest dependence and 5 the weakest. Plants with different longevity were classified as short-lived (annuals, biennials or monocarpic perennials – shown in gray) or perennials (shown in black). Error bars show means \pm 1 SE; n is the number of species in each group. Effect sizes were transformed with Fisher's Z algorithm.

following the Adams' approach. Phylogenetic meta-analysis did not identify a result significantly different from zero in the overall effect of plant size on flowering time. However, regarding this effect, the ordinary meta-analysis was favoured with lower AIC. No analysis or approach leads to a significant overall effect of pre-dispersal seed predation on flowering onset (Table 3). The AIC was lower in the ordinary than in the phylogenetic meta-analysis in the case of pre-dispersal seed predation using the two approaches (Adams' & Lajeunesse's) (Table 3).

DISCUSSION

Incidence and strength of phenotypic selection on flowering phenology

After reviewing currently available evidence of phenotypic selection on flowering time and synchrony, we identified interesting patterns of

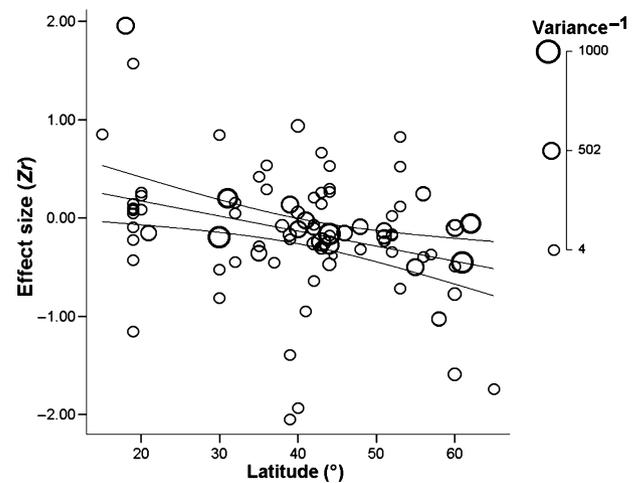


Figure 3 The relationship between selection on flowering time (effect size) vs. latitude. The effect sizes were transformed into Fisher's Z -values to improve normality. Lines show the fitted regression line and its 95% confidence interval. The size of bubbles is proportional to the inverse of the variance of the effect size.

selection on flowering phenology, the most significant of which is that phenotypic selection tends to favour early flowering plants at higher latitudes. No less importantly, we did not find a consistent pattern of selection on flowering synchrony. That we identified consistent evidence of selection in one component of phenology but not in the other suggests that the identity of the phenological trait under selection is an important consideration in studies like ours. Some previous studies have shown that selection can be strong on some organismal traits but not on others; reviews where the exact identity of the target trait of selection is unknown (e.g. Hoekstra *et al.* 2001; Kingsolver *et al.* 2001) may have underestimated the prevalence and strength of selection on some ecologically relevant traits. For a plant to flower earlier than other members of a population may have several advantages if early flowering individuals avoid competition for pollinators, early flowering favours out-crossing, or early reproducing plants have more time for seed maturation and a longer growing season for the progeny resulting from seeds that germinate immediately (reviewed by Kudo 2006 and Elzinga *et al.* 2007). However, the latter likely does not explain our findings because most studies in this meta-analysis did not evaluate recruitment as a metric of fitness. Although phenotypic selection on flowering time seems to be weak ($Zr = -0.14$), one should keep in mind that the data set comprises a broad range of species with contrasting life forms and habitats. Also, in contrast to previous reviews on phenotypic selection (Hoekstra *et al.* 2001; Kingsolver *et al.* 2001) we considered the direction of selection instead of absolute values and used a far more conservative approach: the phylogenetic meta-analysis (Adams 2008; Lajeunesse 2009). Therefore, even though the reported Zr could be low, it has a large biological significance because it reflects a consistent (in strength and direction) phenotypic selection on flowering time in species in many different regions of the world.

Although previous comparative studies looking at phenology demonstrated that phylogenetic membership strongly affects flowering time (Kochmer & Handel 1986; Johnson 1993), in our study the AIC favoured the results of ordinary meta-analysis over the phylogenetic meta-analysis (Table 1). This means that phylogenetic information does not improve model fit and thus, there is not a

Table 3 Overall effect sizes of the influence of plant size (size) and pre-dispersal seed predation (predation) on flowering time. Two approaches (Adams 2008 and Lajeunesse 2009) and analyses (ordinary meta-analysis and phylogenetic meta-analysis, in table 'ordinary' and 'phylogenetic' respectively) were carried out. The estimated effect sizes, their standard errors and associated statistics are shown. The AIC is presented to assess model fit

Approach	Effect	Analysis	Estimate \pm 1 SE	Statistics (Z)	AIC
Adams	Size	Ordinary	-0.247 \pm 0.150	-1.652 ns	62.805
		Phylogenetic	-0.135 \pm 0.898	-0.151 ns	108.197
	Predation	Ordinary	-0.101 \pm 0.074	-1.362 ns	9.223
		Phylogenetic	-0.162 \pm 0.248	-0.652 ns	23.448
Lajeunesse	Size	Ordinary	-0.247 \pm 0.122	-2.017*	85.17
		Phylogenetic	-0.244 \pm 0.161	-1.516 ns	119.65
	Predation	Ordinary	-0.088 \pm 0.051	-1.726 ns	50.82
		Phylogenetic	-0.069 \pm 0.059	-1.166 ns	59.16

* $P < 0.05$.

ns, non-significant result.

significant phylogenetic signal in selection on flowering time in this data set. This contrast between our study and previous research may be because flowering time alone and selection on flowering time are very different biological phenomena. In addition, our study considered a largely different set of species than that of previous work.

Selection on flowering synchrony had an overall trend not significantly different from zero and no moderator variable significantly accounted for variation in selection on this descriptor of phenology. This is perhaps because the advantage, disadvantage or neutrality of blooming synchronously is case specific. Flowering synchronously can improve pollinator attraction or satiate predators in some species (Augspurger 1981). However, it is far from being a rule. Flowering synchrony can also negatively affect plants pollinated by deceit (Parra-Tabla & Vargas 2004; Sun *et al.* 2009), whilst asynchrony may encourage the movements of pollinators and seed-dispersing animals (Rathcke & Lacey 1985; Melampy 1987; Marquis 1988). Also, multiple factors may have affected flowering synchrony and contributed to nullify an overall trend in selection on synchrony. For example, Gómez (1993) found that selection on flowering synchrony imposed by pollinators and herbivores in *Hormathophylla spinosa* L. was opposing and showed spatio-temporal variation, resulting in a lack of phenotypic selection on synchrony. Likewise, Ollerton & Lack (1998) found that flowering synchrony of *Lotus corniculatus* individuals was positively correlated with increased seed predation in some years but not others. Additionally, the fact that selection on flowering synchrony is a case of multilevel selection (Parra-Tabla & Vargas 2007) makes this a more context dependent trait than other phenological traits at an individual level, leading to greater among-study variation and finally the inconsistent overall trend we have found.

Factors affecting selection on flowering phenology

The heterogeneity seen in selection on flowering time was in general high; this variability was significantly explained by latitude and by the duration of the reproductive event (Table 2). Several abiotic environmental variables are associated with latitude; for example, the length of the growing season and suitable conditions for seed maturation and seedling survival are shorter at higher latitudes. Therefore, abiotic conditions (e.g. frost, shade from canopy closure) sometimes lethal for

young plants living at highest latitudes could be the reason why temperate plants are under stronger selective pressure for flowering earlier. Although there is also appreciable seasonality in tropical ecosystems, these are mainly linked to water availability, though many species in seasonal tropical dry forest bloom during the dry season (Bullock 1995), suggesting that drought in the tropics is not a strong limiting factor for plant flowering. It is expected that duration of flowering season reflects the length of favourable conditions for reproduction; following this rationale one would expect stronger phenotypic selection on time of flowering for plants with brief flowering seasons. Although our result supports this prediction, it should be treated with caution as the effect of duration of the flowering season was actually weak and is not easily interpretable because it was only significant after accounting for phylogenetic relatedness among plant species (Table 2).

Pollinator-mediated selection has been invoked as a strong evolutionary force acting on flowering time because pollinators tend to favour earlier flowering plants (Elzinga *et al.* 2007). However, significant pollinator-mediated selection has only been tested experimentally under field conditions for rather few species (Sandring & Ågren 2009; Parachnowitsch & Kessler 2010). If pollinators are indeed strong selective agents we could predict that phenotypic selection may be weaker in less pollinator-dependent plants or in wind-pollinated species. However, contrary to this prediction we found that less dependent plants are subject to stronger selection and no effect of pollen vector (wind or animal) on selection on flowering time (Table 2). This result was supported by both ordinary and phylogenetic meta-analyses. It is possible that this unexpected result could be due to the disproportional number of annual plants among less pollinator-dependant plants (Fig. 2), because these plants are subject to stronger selection than perennials (see ordinary meta-analysis results in Table 2). Therefore, these factors (dependence on pollinators and longevity) are confounded to some extent. Unfortunately analysis of the interaction between these two factors was not possible because there are not enough short-lived plants in categories 1 and 2 of the dependence on pollinator factor and because of computational limitations in phylogenetic meta-analysis.

Unlike polycarpic perennials, lifetime fitness in annuals and monocarpic perennials relies on only one reproductive season; therefore, to flower during times of favourable conditions should be a more critical issue for these plants. Also, selection on flowering time might vary between reproductive seasons (Dominguez & Dirzo 1995; Ollerton & Lack 1998; Ollerton & Diaz 1999; Parra-Tabla & Vargas 2004); therefore, it is not difficult to think that lifetime fitness in plants with multiple reproductive events (polycarpic perennials) is less affected by flowering time in a particular year compared to annuals or monocarpic perennials. Certainly, this prediction was favoured for the results of the ordinary meta-analysis. However, AIC indicates that the best model is the phylogenetic meta-analysis where no significant effect of this factor was found (Table 2). This suggests that with species included in our data set, the effect of longevity is confounded with phylogeny.

Effects of plant size and pre-dispersal seed predation on flowering time

Flowering time may be restricted by the need for sufficient time for vegetative growth and resource accumulation before flowering; therefore, larger plants are expected to flower earlier (Lacey 1986;

Houle 2002) a phenomenon which has been shown in some studies, (e.g. Ollerton & Lack 1998). Our analysis shows a weak trend for larger plants to flower earlier, as expected (Table 3). One possibility we cannot discard with the current data set is that flowering time and size are genetically correlated characters and selection on size is working indirectly on flowering time. One way to check for indirect selection on flowering time is by comparing selection differentials and selection gradients on flowering time when size is considered in the regression model. However, this kind of information is available for only a very limited number of species (e.g. *Arabidopsis lyrata*; Sandring *et al.* 2007), precluding any generalizations.

It has been suggested that flowering time might have evolved via selection on post-flowering events such as timing of fruit production and a trend seen in previous studies suggests that seed predators tend to favour off-peak or late flowering individuals (Elzinga *et al.* 2007). However, when we analysed the evidence available at the moment, the effect of pre-dispersal seed predators on flowering time was consistently non-significant in all our analyses (Table 3). Even so, we should recognize that the number of species is small and therefore the effect of pre-dispersal seed predation on flowering time deserves further evaluation when more studies become available.

PERSPECTIVES AND CONCLUSION

There is enough evidence available to conclude that phenotypic selection tends to favour early flowering plants. Contrary to previous suggestions, pollinators and seed predators do not generally impose consistent selection on flowering times. Instead, environmental conditions associated with latitude seem to be more important moderators of phenotypic selection on flowering time. This has clear implications for the future evolution of temperate floras under scenarios of global climate change (e.g. Jump & Peñuelas 2005). For example, we might predict that as northern hemisphere springs become earlier in some parts of Europe or North America (e.g. Miller-Rushing & Primack 2008; Schleip *et al.* 2009), selection for earlier flowering individuals may become stronger.

The strength and prevalence of phenotypic selection varies between biological traits and deeper insights into patterns of phenotypic selection are likely to emerge if the exact identities of the traits targeted by selection are taken into account in future studies, as we found once we had differentiated between phenological descriptors. In the future, more studies looking at direct and indirect phenotypic selection on flowering time as well as herbivore mediated selection on phenological traits are needed for a better understanding of their importance in influencing the evolution of flowering phenology. Using phylogenetic meta-analysis to assess how moderator variables affecting phenotypic selection interact with one another is another promising area for future research but requires some computational and software improvements before it can be done. Research on phenotypic selection is rapidly accumulating and this, coupled with statistical advances, will enable us to perform meta-analyses with greater power, allowing us to determine the strength and incidence of phenotypic selection in nature.

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SUPPORTING INFORMATION

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

Appendix S1 Species and family list, effect sizes and moderator variables used in the meta-analyses.

Appendix S2 Authorship, title and references of original sources of data.

Figure S1 Funnel plots of random-effects meta-analyses fitted to assess the effects of: selection on flowering time, selection on flowering synchrony, plant size on flowering time and pre-dispersal seed predation on flowering time. Symmetry of data suggests no publication bias.

Figure S2 Forest plots of random-effects meta-analyses fitted to assess the effects of: selection on flowering time, selection on flowering synchrony, plant size on flowering time and pre-dispersal seed predation on flowering time. Each row shows per species effect size and its standard error.

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